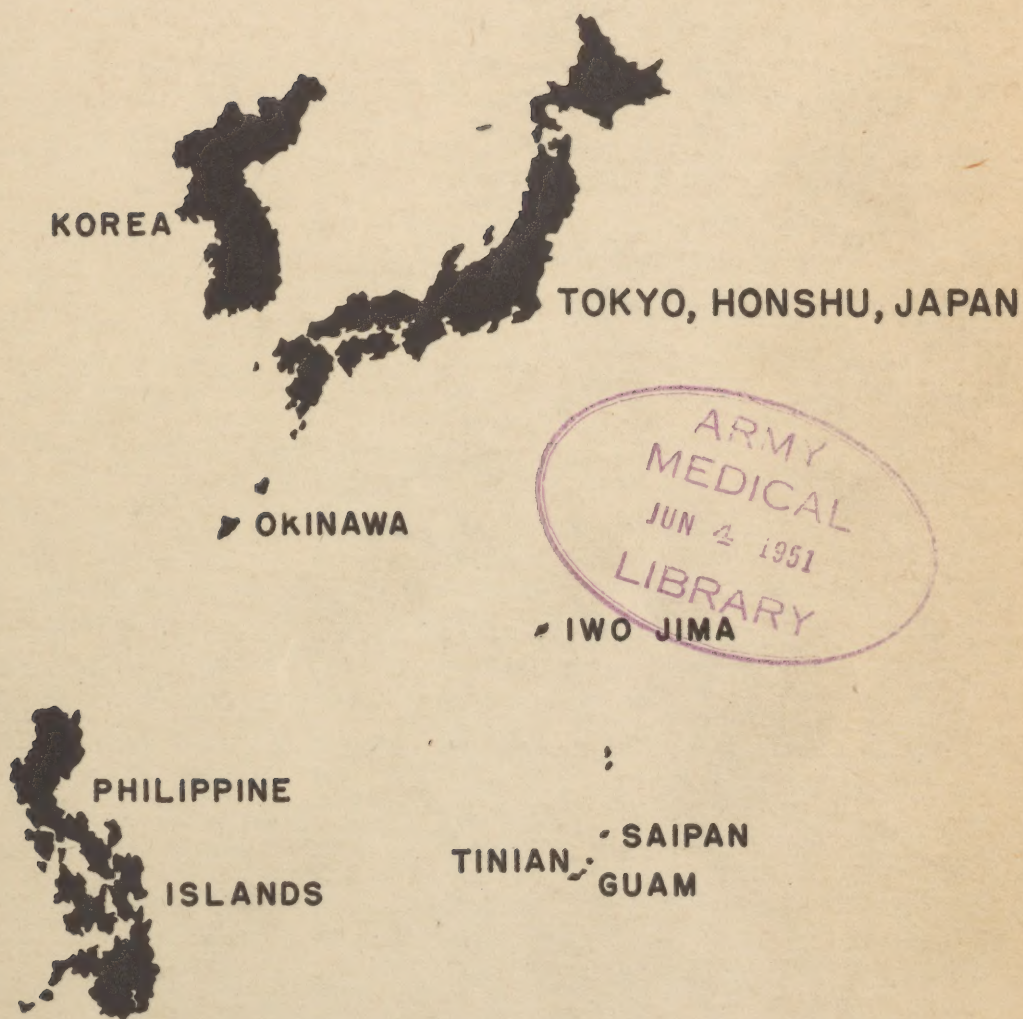


406 MEDICAL GENERAL LABORATORY

ANNUAL HISTORICAL REPORT

1950



PROFESSIONAL SECTION

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PREFACE

The year 1950 saw a transition from peace to war, and with it a marked increase in all aspects of the duties and responsibilities of this Laboratory. While this report could well stand merely as representative of what can be accomplished by loyal and hardworking individuals of each Department under such increased pressure, it is designed primarily for two more general purposes.

The war has brought many Medical Service personnel to this theater for the first time and it is hoped this report may serve to call to their attention some of the more important health problems, against which it is their problem to guard our troops.

Secondly, it is hoped that this report will serve to emphasize the fact that in the midst of a sudden and unexpected conflict, there must continue an honest effort to broaden and increase medical knowledge - again to improve the role of the Medical Service in maintaining the combat strength of our military defenses.

This report records many activities of this unit in fulfilling its theater mission "to supplement the epidemiologic, sanitary, and diagnostic services available in other medical department laboratories, and to investigate outbreaks of disease and conditions which affect, or may affect the health of persons ... of the command".

R.L.H.

ACKNOWLEDGEMENT

In all our efforts the success attained has been due to the excellent cooperation and counsel available and offered from units and headquarters of all branches of the Armed Forces of this theater, individual officers in medical installations of the Far East Command, individual Japanese and American scientists, numerous Prefectural and other health agencies, higher echelons of military medical service, Public Health and Welfare Section (SCAP), Ministry of Welfare, National Institutes of Health (Japan and U.S.), Institute of Infectious Diseases, Institute of Public Health, Research Institute of Natural Resources, Yamanashi Medical Research Institute, Kitasato Institute, Okayama University Medical School, Tokyo University, To-Ho Pharmacological University, Institute of Psychiatric Diseases, American, Japanese, and other Red Cross representatives, blood donors of all nationalities, Ueno Park Zoological Gardens, and many others.

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DEPARTMENT OF MEDICAL ZOOLOGY

A summary of all diagnostic examinations and tests completed during the current year appears in Table I.

The data summarized in Table I will be discussed in detail below.

Table I. Summary of Diagnostic Procedures

	Number of <u>Specimens</u>	Number of <u>Examinations</u>
Examination of Feces		
Routine	4,647	5,377
Chemotherapy study	2,279	10,804
Epidemiologic surveys	2,604	5,382
U. S. Casualties from Korea	95	95
Skin test studies	340	742
Testing technical methods	127	409
Appendiceal contents	587	587
Subtotal	10,679	23,396
Examination of Blood Smears for		
Malaria	503	835
Filariasis	454	902
Subtotal	957	1,737
Examination of Sputum for Paragonimiasis	50	50
Examination of Perianal Imprints for Enterobiasis	558	558
Examination of Urine for Gonadotropic Hormone	751	1,159
Miscellaneous identifications	31	31
Grand Total	13,026	26,931

Inasmuch as studies on schistosomiasis constitute a sizeable portion of special projects undertaken by this Department, relatively large numbers of Oncomelania nosophora, the intermediate host, and related snails were studied or utilized as follows.

Table II. Summary of Snails Used for Experimental Studies

Seasonal Studies on <u>Schistosoma japonicum</u> in the Snail	
Kofu	22,011
Kyushu	3,437
Schistosome Dermatitis Studies with the Snails	
<u>Polypylis hemisphaerula</u>	16,154
Snails collected in "Operation Santobrite"	5,137
Snails used in Molluscicide Screening	15,000
Snails used in Biological Studies	2,000
Snails collected in Surveys	2,536
Snails crushed in Preparing Cercarial Antigen	5,000
Snails crushed for Cercariae in Animal Infections	25,000
Total	96,275

Routine

SUMMARY OF STOOL EXAMINATION OF JAPANESE AND AMERICAN PERSONNEL: A total of 2,042 Japanese Nationals, most of whom were employed as servants or food handlers in the Tokyo area, received stool examinations for intestinal parasites. Of this number, 75% were parasitized; 69.1% harbored helminths and 22.1% protozoa. American personnel numbering 2,604 were also examined routinely for intestinal parasites, 25.5% of these being parasitized. Ten per cent carried helminths, while 16.7% were infected with protozoa. The incidence of intestinal parasites in Americans is much less than in the Japanese. The data are summarized in Table III.

Table III. Parasitism in Japanese and Americans Examined Routinely by Stool During 1950

	Japanese		Americans	
	Number	% Infected	Number	% Infected
No. persons examined	2042		2605	
No. parasitized	1531	75.0	664	25.5
No. with helminths	1411	69.1	261	10.0
No. with protozoa	452	22.1	437	16.7
Helminths:				
<u>Ascaris lumbricoides</u>	958	46.9	174	6.7
<u>Trichuris trichiura</u>	504	24.7	49	1.9
Hookworm	359	17.6	40	1.5
<u>Trichostrongylus</u> sp.	319	15.6	8	
<u>Strongyloides stercoralis</u>	3		6	
<u>Clonorchis sinensis</u>	9		3	
<u>Metagonimus yokogawai</u>	0		0	
<u>Enterobius vermicularis</u>	9		8	
<u>Hymenolepis nana</u>	3		0	
<u>Taenia</u> sp.	0		1	
<u>Schistosoma japonicum</u>	5		0	
Protozoa:				
<u>Endamoeba histolytica</u>	45	2.2	66	2.5
<u>Endamoeba coli</u>	264	12.9	197	7.6
<u>Endolimax nana</u>	212	10.4	194	7.4
<u>Iodamoeba butschlii</u>	14	0.7	18	0.7
<u>Giardia lamblia</u>	48	2.4	75	2.9
<u>Chilomastix mesnili</u>	3		2	
<u>Enteromonas hominis</u>			1	
<u>Trichomonas hominis</u>			2	

PARASITES RECOVERED FROM CONTENTS OF SURGICALLY REMOVED APPENDICES: During 1950 the contents from a total of 587 appendices were examined as part of a joint project with the Department of Pathology. Of these 587 specimens, only 14, or 2.4% were infected. As will be seen from Table IV, there were almost twice as many helminths as protozoa encountered.

Table IV. Identification of Parasites from Contents of Surgically Removed Appendices During 1950

	Number	% Infected
Total specimens examined	587	
No. with parasites	14	2.4
No. with helminths	9	1.5
No. with protozoa	5	0.85
Helminths:		
<u>Ascaris lumbricoides</u>	7	
<u>Trichuris trichiura</u>	1	
<u>Enterobius vermicularis</u>	1	
<u>Hymenolepis nana</u>	1	
Protozoa:		
<u>Endamoeba coli</u>	2	
<u>Giardia lamblia</u>	3	

STUDIES ON SCHISTOSOMIASIS: Since 1947 a continuous program has existed aimed at the collection of basic facts, and the study of methods for improving diagnosis and the control of schistosomiasis japonica. Although considerable progress has been made during the past three years this account is regarded in the nature of a progress report.

"OPERATION SANTOBRITE" - A SCHISTOSOME SNAIL ERADICATION PROGRAM IN JAPAN: In 1947 the Department of Medical Zoology with the cooperation of the Japanese National Institute of Health undertook to screen in the laboratory a number of potential molluscicides supplied by various chemical manufacturers in the United States. The best ones were further tested on small field plots at Kofu (1,3) with the cooperation of the Yamashiro Medical Research Institute and other Prefectural officials. Two chemicals gave promising results, namely Santobrite (79% sodium pentachlorophenate), DNI (40% dinitro-o-cyclohexylphenol). To determine whether snail control was practicable, it was imperative that large scale field trials of one of the most promising chemicals be undertaken. The Monsanto Chemical Company generously furnished a ton of Santobrite for this experiment in Japan.

Methods - Nagatoishi-cho in Fukuoka Prefecture on the island of Kyushu was selected for this project. The area comprised 150 acres which supported a population of 1050 persons, and was enclosed by dykes. This community was selected for the study because the people and fields were relatively isolated and consequently there was less danger of introducing infected snails from outside sources. The people of this area were 73% infected both in 1948 and 1950. Since 1948 treatments have somewhat reduced the intensity of the infections in the human population, although the incidence remained the same and the snails continued to be quite heavily infected.

It was decided to apply the chemical bi-annually, in the spring and the fall. In the spring a total of 170 square foot quadrats were selected at random and marked by stakes (Fig. 1). All snails were collected from each quadrat, counted, and the per cent of living snails determined. Upon completion of these pretreatment counts, Santobrite was applied and two weeks later post-treatment counts were made in adjacent quadrats. Snails were collected with great care, since dead snails were more easily overlooked than live ones. The per cent reduction in snail population was then calculated. Examination of this method revealed that a truer picture of the results would be obtained if calculations were based only upon the numbers of viable snails recovered each time, since this would eliminate possible errors due to the inherent difficulties of detecting dead snail shells and of determining how long they had been dead. During the fall 20 new quadrats were added. These stations were located beyond the ditches, usually at the edge of rice paddies. The controls consisted of an untreated area where snails were collected from 12 quadrats.

Voluntary labor for six crews was furnished by the local Japanese. Each team consisted of a foreman interpreter, chemical mixer, at least four water carriers, five sprayers, and five grass cutters. Each team was supplied with three sprinkling cans and two Lofstrand pressure sprayers. The latter were used to reach under culverts, overhanging banks and into rodent or other type burrows. In addition to the sides of the ditches, the water in the ditches and the tops of the banks were also sprayed.

Santobrite (390 gm.) was dissolved in 20 gallons of water giving a dilution of approximately 1:200 and applied at the rate of roughly 390 mg. per square foot. The length of ditch sprayed varied between 150 and 200 feet, depending upon the width of the ditch.

Spring Treatment - The initial pre-treatment collection made in March, 1950 yielded a total of 2,436 snails of which 2,364, or 97%, were viable. This figure of 2,364 viable snails constitutes the base line for future comparisons. Santobrite was then applied and when the post-treatment collections were made some two weeks later in early April only 43, or 3%, of the 1,433 snails that were collected were alive. This represents a population reduction of 98.14% of the viable snails (Table V). The counts of the controls revealed an increase in dead snails of 9% when the post-treatment counts were compared with the pre-treatment ones (Table V). The increase in the ratio of dead to living snails in the treated area in comparison to that in the controls was quite significant.



FIGURE 1 — MAP OF NAGATOISHI-CHO SHOWING
THE AREA OF "OPERATION SANTOBRITE"

Table V. Summarized Results of Bi-Annual Molluscicide Application,
Nagatoishi-Cho, 1950

Period	Pre-treatment Counts				Treated Area	Post-treatment Counts			
	No. of Quadrats	No. Alive	No. Dead	Total	% Reduction in Living Snails	No. Alive	No. Dead	Total	% Reduction in living Snails
Spring	170	2364	72	2436	--	43	1300	1433	98.14*
Fall	190	568	34	602	76*	234	432	666	90.2 *

					Control				
					% Dead				% Dead
Spring	12	80	2	82	2.4	62	8	70	11.4
Fall	12	109	13	122	10.6	97	1	98	1

✓ Included 20 new quadrats along the edges of paddies, labelled pl-p20 on
Figure 1.

* Based on the living snails in the first pre-treatment count.

In checking the effectiveness of the spraying in the spring a number of additional quadrats were examined which were not in the treated areas. Most of these new quadrats were near the edges of, or under piles of straw in the paddies. Since some viable snails were encountered, it was decided to add 20 new quadrats in the fall to cover such areas along the edges of the paddies.

Fall Treatment - The pre-treatment counts were made in early October of 1950, at which time a total of 568 viable snails were recovered. This was a marked increase over the 43 living snails found at the time of the spring post-treatment count. The ditches and edges of the paddies were then sprayed with Santobrite. When the post-treatment counts were made in early November of 1950, 234 viable snails were recovered. This represents a 90.2% reduction in the viable snail population over the original spring pre-treatment collection figures (Table V).

Only 10, or 4.3% of the 234 viable snails collected during the fall post-treatment period were infected with S. japonicum (Table VI). Snails 7 mm and 7.5 mm in length had the highest per cent of infection, namely 7% and 5% respectively.

Table VI. Summary of Data on Snails Collected After the Fall
Application of Sodium Pentachlorophenate (Santobrite) 1950

Size of Snail in mm.	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5
No. Collected	1	3	3	0	11	20	108	144	139	126	97	14
No. Alive						2	28	40	52	57	50	5
% Alive	0	0	0	-	0	10	35.9	27.8	37.4	45.2	51.5	35.7
% of Total in each Group	.15	.45	.45	-	1.6	3	16.2	21.6	20.8	18.9	14.5	2.1
No. Infected	-	-	-	-	-	0	2	2	2	1	2	1
% of Infection	-	-	-	-	-	0	7.1	5	3.8	1.8	4	20

Discussion - While this constitutes a remarkable achievement from the public health standpoint in the practical control of snails in such a large area, it was disappointing because of the thirteen-fold increase in the number of snails found when the fall pre-treatment tabulations were made compared with the spring post-treatment figures.

How can one explain the differences in the results and the apparent increase in the number of snails encountered? A satisfactory explanation of these findings is not at hand. It cannot be argued that the increase was due to the additional 20 quadrats, as only 33 viable snails were taken from these in the fall (Tables VII and VIII). It seems more likely either that all of the snails were not out of hibernation at the time of the post-treatment counts in April or that new snails were introduced from the river outside of the dykes, through the pump when it was filling the irrigation ditches during the summer. The latter is a possible explanation, since colonies of Oncomelania nosophora are known to exist upstream from the inlet canal leading to the pump and local records reveal that periods of flooding usually occur during the summer months. At such times areas where these colonies lie are flooded, thus making it possible for them to be swept into the inlet canal and so through the pump and into the otherwise protected irrigation ditches of Nagatoishi-cho (Fig. 1). Further evidence to support this hypothesis comes from the fact that only in the fall were viable snails recovered near the pump house in the main, concrete lined irrigation ditch, an area which had been completely devoid of snails at the time of the spring pre-treatment counts. In addition the greatest numbers of snails appeared in the main transverse ditches (Fig. 1, Tables VII and VIII) which are fed directly from the main, concrete lined ditch (see "I" on Fig. 1).

Still another explanation of this increase which might be carefully considered and checked during 1951 lies in the possibility that these snails may be the offspring of those which survived the spring treatment. Data appearing elsewhere in this report, v.i. Seasonal Studies, and as previously noted by Sugiura (4) indicate the possibility that snails hatching in June-July reach a length of 6.5 to 9 mm. with seven to eight whorls by the end of October or November. The measurement of the 666 living and dead snails collected after the fall treatment also conform to this pattern (Table VI), thus supporting the hypothesis that many of these snails were of this year's brood. Such an interpretation would account for the increase of snails in the fall pre-treatment counts. Obviously, steps will have to be taken during 1951 to determine which of these hypotheses fits the facts.

It will be noted from Table V that 234 viable snails were collected at the time of the fall post-treatment counts. This seems like an unusually large number and suggests that the application of Santobrite was not as effective in the fall as in the spring, and raises the question of why not. Several possible factors might account for this. In spite of the fact that the grass in the treated area was cut before the application of the chemical it is possible that the vegetation was still dense enough to furnish considerable protection to the snails, thus preventing proper contact of the chemicals. Furthermore, the cool, overcast weather may have reduced the activity of the snails causing them to enter a pre-hibernation stage. In such cases the operculum would probably be closed. Considerable rain fell during the period of application of the chemical, thus introducing a third factor which might have influenced the effectiveness of the chemical. In any event it should be borne in mind that the elimination of the last remaining elements of the snail population will become more difficult as the total population is reduced.

Summary - During the first year of treatment of the irrigation ditches in the Nagatoishi-cho project with sodium pentachlorophenate (Santobrite) the snail population was reduced 90.2%. This is sufficiently promising to warrant expressing the belief that complete eradication of the snails in this area may be approached by the end of 1951.

LABORATORY SCREENING OF MOLLUSCACIDES: Laboratory screening tests for molluscicides were performed on 132 organic compounds secured with the cooperation of the Division of Tropical Diseases of the National Institute of Health at Bethesda, Maryland, and five received direct from the Dow Chemical Company. The method follows that previously used for screening tests by this laboratory in 1949. Briefly, the procedure consisted of exposing ten adult, living snails in petri dishes to single filter paper inserts saturated with dilutions of 1:1000, 1:5000 and 1:10,000 of the chemical being tested. At the end of 96 hours, the number of snails destroyed is determined and the LD₅₀ calculated according to the method of Reed and Muench (2). Those compounds having no lethal effect in the above dilutions are considered to be unsatisfactory and are eliminated from further study. Chemicals destroying snails in any or all of the

Table VII. Snail Counts by Area Before and After Treatment With Sodium Pentachlorophenate (Santobrite) in the Spring of 1950

Treatment Area	No. of Quadrats	Pre-treatment, 21-23 March			Post-treatment, 15-17 April		
		No. Alive	No. Dead	Total	No. Alive	No. Dead	Total
A	7	100	1	101	3	46	49
B	8	63	10	73	10	82	92
C	9	130	6	136	4	122	126
D	9	72	1	73	2	101	103
E	10	115	0	115	2	68	70
F	8	159	9	168	1	63	64
G	4	112	1	113	2	59	61
H	4	0	1	1	0	1	1
O	7	254	5	259	4	133	137
I	14	161	4	165	2	103	105
II	6	85	4	89	0	62	62
III	11	86	1	87	0	50	50
IV	12	142	2	144	3	83	86
V	12	156	3	159	1	66	67
VI	14	205	12	217	1	51	52
VII	10	34	1	35	0	68	68
VIII	11	270	9	279	3	112	115
IX	7	147	2	149	5	58	63
X	7	83	0	83	0	62	62
Total	170	2,364	72	2,436	43	1,390	1,433
% Dead			2.9			96.9	
Control Area							
1	1	18	0	18	0	2	2
2	1	3	0	3	6	1	7
3	1	11	0	11	8	1	9
4	1	6	1	7	8	1	9
5	1	3	0	3	8	0	8
6	1	3	0	3	1	0	1
7	1	2	1	3	0	1	1
8	1	4	0	4	2	2	4
9	1	11	0	11	4	0	4
10	1	9	0	9	5	0	5
11	1	3	0	3	14	0	14
12	1	7	0	7	6	0	6
Total	12	80	2	82	62	8	70
Percent		97.6	2.4		88.6	11.4	

dilutions are retested in higher or lower dilutions in order to obtain the LD₅₀ and MLD. The LD₅₀ of a compound serves as a rough index of its molluscicidal efficiency and furnishes a basis for determining whether or not the chemical should be given a field plot test. The results of the laboratory tests are summarized in Tables IX. and X.

Summary - One hundred and thirty-seven chemicals were screened in the laboratory for molluscicidal properties against the amphibious snail, Oncomelania nosophora, and only 3 chemicals gave results indicating them to be effective molluscicides. Listed

Table VIII. Snail Counts by Area Before and After Treatment with Sodium Pentachlorophenate (Santobrite) in the Fall of 1950

Treatment Area	No. of Quadrats	Pre-treatment, 3-5 October			Post-treatment, 3-6 November		
		No. Alive	No. Dead	Total	No. Alive	No. Dead	Total
A	7	24	0	24	1	21	22
B	8	49	3	52	21	68	89
C	9	115	9	124	33	45	78
D	9	37	0	37	16	45	61
E	10	42	7	49	8	32	40
F	8	49	1	50	5	12	17
G	4	8	0	8	5	26	31
H	4	1	0	1	0	1	1
O	7	3	2	5	7	5	12
I	14	6	0	6	1	4	5
II	6	1	0	1	1	10	11
III	11	12	1	13	4	4	8
IV	12	22	0	22	25	10	35
V	12	17	1	18	4	25	29
VI	14	25	3	28	17	24	41
VII	10	12	0	12	4	15	19
VIII	11	59	2	61	20	48	68
IX	7	75	3	78	26	20	46
X	7	2	1	3	3	3	6
P	20	9	1	10	33	14	47
Total	190	568	34	602	234	432	666
Percent		94.4	5.6		35.1	64.9	

Control Area

1	1	15	1	16	2	0	2
2	1	1	2	3	6	0	6
3	1	10	0	10	3	0	3
4	1	13	1	14	3	0	3
5	1	1	0	1	0	0	0
6	1	0	0	0	0	0	0
7	1	3	2	5	10	0	10
8	1	13	3	16	11	1	12
9	1	1	1	2	7	0	7
10	1	8	1	9	9	0	9
11	1	7	1	8	40	0	40
12	1	37	1	38	6	0	6
Total	12	109	13	122	97	1	98
Percent		89.4	10.6		99	1	

in order of practical merit they are: Dowcide B (Sodium trichlorophenate 85%), 2, 4, 5-trichlorophenol and Dowcide G (Sodium pentachlorophenate 75%, sodium salts of other chlorophenols 13%). These will require further testing in the field.

Table IX. Results of Laboratory Tests Obtained by Exposing Groups of 10 Snails
(*Oncomelania nosophora*) for 96 hours to Various
Potential Molluscacides During 1950

<u>Chemical No.</u>	<u>Chemical or Trade Name</u>	<u>Solvent Used</u>	<u>Source</u>
LD ₅₀ Secured at Dilution between 1:40,000 and 1:56,000			
1	Dowcide 31	Water	Dow Co.
553	2, 4, 5-Trichlorophenol	Ether	NIH
LD ₅₀ Secured at Dilution between 1:20,001 and 1:26,000			
2	Dowcide 31	Ether	Dow Co.
3	Dowcide G	Water	Dow Co.
4	Dowcide -2-S	Ether	Dow Co.
495	p-tert-Butylphenol	Ether	NIH
180	2-Chloro-6-phenylphenol	Ether	NIH
LD Secured at Dilution between 1:8,001 and 1:14,000			
511	2,4-Dichlorophenol	Ether	NIH
512	2,6-Dichlorophenol	Ether	NIH
543	p-Nitrophenol	Ether	NIH
5	Dowcide F	Water	Dow Co.
6	Copper pentachlorophenate	Acetone	Dow Co.
LD ₅₀ Secured at Dilution between 1:6,001 and 1:8,000			
474	o-Aminophenol, HCL	Water	NIH
490	Bromohydroquinone	Ether	NIH
552	p-Bromophenacyl bromide	Ether	NIH
744	1,5-Dihydroxynaphthalene	Ether	NIH
LD ₅₀ Secured at Dilution between 1:4,001 and 1:6,000			
479	p-tert-Amylphenol	Ether	NIH
502	4-Chloro-3-methyl-2-nitrophenol	Ether	NIH
517	2-Dimethylaminomethyl-4-(1,1,3,3-tetramethylbutyl) phenol	Ether	NIH
531	Hydroquinone monomethyl ether	Water	NIH
738	1-Hydroxy-2-naphthaic acid	Water	NIH
750	4,4'-Dihydroxydiphenylsulfone	Ether	NIH
LD ₅₀ Secured at Dilution between 1:2,001 and 1:4,000			
77	Hexachlorophenol	Ether	NIH
131	S-((Beta-(p-tert-Octylphenoxy-Beta-ethoxy) ethyl)) thiopseudourea, HCl, hemihydrate	Water	NIH
156	Diphenylnitrosamine	Ether	NIH
501	Chlorohydroquinone	Ether	NIH
513	m-Diethylaminophenol oxalate	Alcohol	NIH
519	2,6-Dinitro-4-chlorophenol	Ether	NIH
789	3-Phenylsalicylic acid	Ether	NIH
801	p-Isopropylphenol	Ether	

Table IX. Continued

<u>Chemical No.</u>	<u>Chemical or Trade Name</u>	<u>Solvent Used</u>	<u>Source</u>
LD ₅₀ Secured at Dilution between 1:1,001 and 1:2,000			
157	2,4,6-Tribromoanisole	Ether	NIH
455	Acetyl-m-aminophenol	Ether	NIH
522	1,3,5-Ethylmethylphenol	Water	NIH
545	p-Nitrophenol, sodium salt	Ether	NIH
788	4-Phenylcatechol	Ether	NIH
LD ₅₀ Secured at Dilution of 1:1,000 or below			
44	3-Methyl-1, 4-naphthoquinone	Ether	NIH
186	2,4-Dinitrobromobenzene	Ether	NIH
246	Pentabromophenol	Acetone	NIH
406	1-Dodecylguanidine acetate	Ether	NIH
489	p-Benzylphenol	Ether	NIH
505	o-Cyclohexylphenol	Ether	NIH
506	Diacetyl-p-aminophenol	Water	NIH
508	2,6-Dibromo-4-nitrophenol	Ether	NIH
510	2,6-Di-tert-butyl-4-methylphenol	Ether	NIH
518	p-Dimethylaminophenol sulfate	Water	NIH
526	Hydrindylphenol	Ether	NIH
538	m-Nitroacetophenone	Ether	NIH
556	2,4,6-Tribromoaniline	Ether	NIH
575	2-Bromoacenaphthene	Ether	NIH
582	d-Bromocamphor	Ether	NIH
736	1-Naphthol-4-sulfonic acid	Water	NIH
740	Di-Beta-naphthol	Ether	NIH
743	Beta-Naphthol	Ether	NIH
748	p-Hydroxydiphenyl	Ether	NIH
749	4,4'-Dihydroxy-3,3'-dimethyldiphenyl	Ether	NIH
751	p,p'-Biphenol	Ether	NIH
753	m-Nitrophenol	Water	NIH
797	p-Hydroxybenzophenone	Ether	NIH
799	2-Chloro-4,6-dinitrophenol	Water	NIH
807	3-Phenylcatechol	Ether	NIH
Compounds Eliminated as Having No Appreciable Lethal Effect at Dilutions of 1:1,000; 1:5,000 and 1:10,000			
19	N-Cyclohexyl-2,3,4,6-tetrachlorophen- oxyacetamide	Ether	NIH
78	2,3,4,6-Tetrachlorophenol	Ether	Dow Co & NIH
158	2-Pivalyl-1,3-indandione	Ether	NIH
160	2-Isovaleryl-1,3-indandione	Ether	NIH
184	Tribromo-Beta-naphthol	Ether	NIH
198	2,3-Dichloro-1,4-naphthoquinone	Acetone	NIH
199	2,4-Dichloro-1-naphthol	Ether	NIH
280	2-Bromo-4-phenylphenol	Ether	NIH
305	o-Nitrophenol	Ether	NIH
454	Acetyl-o-aminophenol	Ether	NIH
456	Acetyl-p-aminophenol	Water	NIH
457	Acetyl-o-methylaminophenol	Ether	NIH
458	Acetyl-p-methylaminophenol	Alcohol	NIH
459	9-Acetyl-1,2,3,4-tetrahydrophenanthrene	Ether	NIH
460	m-Aminoacetophenone	Ether	NIH
461	p-Aminoacetophenone	Ether	NIH
462	2-Amino-5-diethylaminotoluene-monohydro- chloride	Water	NIH
463	Aminohydroquinone diethyl ether	Ether	NIH
464	Aminohydroquinone dimethyl ether	Ether	NIH

Table IX. Continued

<u>Chemical No.</u>	<u>Chemical or Trade Name</u>	<u>Solvent Used</u>	<u>Source</u>
465	1-Amino-2-naphthol HCl	Ether	NIH
466	2-Amino-4-nitrophenol	Ether	NIH
467	4-Amino-3-pentadecylphenol	Ether	NIH
468	o-Aminophenol	Ether	NIH
469	m-Aminophenol	Water	NIH
470	p-Aminophenol	Alcohol	NIH
471	o-Aminophenol HCl	Water	NIH
472	m-Aminophenol HCl	Water	NIH
473	p-Aminophenol HCl	Water	NIH
475	o-Aminophenol-p-sulfonic acid	Water	NIH
476	Sodium 2,7-anthraquinonedisulfonate	Water	NIH
477	Sodium anthraquinone-Beta-sulfonate	Water	NIH
480	Anthraquinone-Beta-sulfonic acid	Water	NIH
481	Barium salt of phenol-p-sulfonic acid	Water	NIH
482	Benzalacetophenone	Ether	NIH
483	Benzalacetophenonedibromide	Ether	NIH
484	p-Benzalaminophenol	Ether	NIH
485	1,2-Benzanthraquinone	Ether	NIH
486	Benzeneazo-o-cresol	Ether	NIH
487	p-Benzylaminophenol	Ether	NIH
492	Bromohydroquinone	Ether	NIH
507	2,4-Diaminophenol HCl	Water	NIH
509	2,5-Di-tert-butylhydroquinone	Water	NIH
515	4,4'-Dihydroxystilbene	Ether	NIH
520	2,6-Dinitrophenol	Ether	NIH
527	Dibutyl ether of hydroquinone	Ether	NIH
528	Dinitrohydroquinone acetate	Ether	NIH
529	Monomethyl-p-amidophenol sulfate	Water	NIH
530	Hydroquinone monobenzyl ether	Ether	NIH
533	Indophenol	Ether	NIH
534	o-Iodophenol	Ether	NIH
535	1-Methylaminoanthraquinone	Ether	NIH
539	1-Nitroanthraquinone	Water	NIH
540	m-Nitrobenzalacetophenone	Water	NIH
544	o-Nitrophenol, sodium salt	Water	NIH
558	2,4,6-Tribromophenyl-p-toluene-sulfonate	Alcohol	NIH
560	2,3,5-Triiodobenzoic acid	Ether	NIH
562	Triphenylchloromethane	Ether	NIH
563	1,3,5-Tribromobenzene	Ether	NIH
564	Triphenylbromomethane	Ether	NIH
567	1,2,4,5-Tetrabromobenzene	Ether	NIH
571	Tetrachlorohydroquinone	Ether	NIH
572	2-Bromo-3-nitrobenzoic acid	Ether	NIH
573	1,2,4,5-Tetrachlorobenzene	Ether	NIH
576	Bromanil	Ether	NIH
577	9-Bromophenanthrene	Ether	NIH
690	2-Naphthol-7-sulfonic acid	Water	NIH
732	m-Cresyl benzoate	Ether	NIH
735	2-Naphthol-4-sulfonic acid	Ether	NIH
737	1,8-Dihydroxynaphthalene-3,6-disulfonic acid	Ether	NIH
742	Alpha-Naphthol	Ether	NIH
746	Copper 4-chloro-2-nitrobenzoate	Water	NIH
747	Picramic acid	Water	NIH
758	p-Nitrosophenol, sodium salt	Water	NIH
790	o-Cresotinic acid	Ether	NIH
791	1-Phenol-4-sulfonic acid, ammonium salt	Water	NIH
798	5-Phenylsalicylic acid	Ether	NIH
802	5-Bromosalicylic acid	Ether	NIH

Table X. Comparative Data on the Most Effective Molluscacides Screened from 137 Organic Compounds During 1950

Survey No. or Proprietary Name	Compound	Solvent Used	M.L.D.	LD ₅₀
*Dowcide B	Sodium trichlorophenate 85%	Water	1:40,000	1:56,340
553	2,4,5-Trichlorophenol	Ether	1:30,000	1:41,000
*Dowcide G	Sodium pentachlorophenate 75%	Water	1:20,000	1:25,760
*Dowcide 31	Chloro-o-phenylphenol 85%	Ether	1:250	1:23,980
495	p-tert Butyl phenol	Ether	1:1,000	1:22,430
*Dowcide-2-S	2,4,6-Trichlorophenol 90%	Ether	1:1,000	1:20,510
180	2-Chloro-6-phenylphenol	Ether	1:2,000	1:20,620
Dow	Copper pentachlorophenate	Acetone	1:250	1:13,230
511	2,4-Dichlorophenol	Ether	1:1,000	1:10,000
543	p-Nitrophenol	Ether	1:250	1:10,000

* An amount of the chemical was used to yield 0.1 gram of the active ingredient in the 1:250 stock solution.

SEASONAL STUDIES OF SCHISTOSOMA JAPONICUM IN THE INTERMEDIATE SNAIL HOST, ONCOMELANIA NOSOPHORA: Introduction - Monthly collections of the snail, Oncomelania nosophora, were made from October, 1947 to October 1949, at four circumscribed areas in Yamanashi Prefecture by McMullen (1,3,8,9). His data afforded information regarding propagation, growth and life-span of the snail; variations in the incidence of Schistosoma japonicum in the snail in the Kofu area; infectivity of snails on the basis of age and sex; and time of occurrence, maturation and duration of the infections in the snail. The findings were both informative and provocative, and it was agreed by all concerned that the study could be profitably continued. The following is a report of the investigations as projected through 1950.

Evaluation of the Collecting Stations and General Findings: Mutsusawa - This has been the most informative of the collecting stations. The data, however, were not collected at the same location as those previously reported (8,9) at Mutsusawa, due to a depletion of the colonies of snails. The present colony has had an incidence of approximately 10% during 1950, whereas it was less than 0.5% for the original colony during the last six months of 1949, and as shown by occasional checks, continued at this low level during 1950 in that original colony.

During March of the current year all collecting sites were subdivided into sharply delineated substations "A", "B", and "C". Whereas Shimosanjo and Aburakawa substations consisted of equal parts of small linear ditches of about 60 meters, Mutsusawa substations were comprised of either one or two narrow paddies located on low terraces of a steep hillside. The breakdown of the Mutsusawa colonies into smaller subdivisions was carried out to determine whether or not the status of the infection varied within these colonies. The Mutsusawa monthly collections were made at "A", "B", and "C" from December 1949 through September 1950. Then for the three subsequent months only "C" was collected in October, "A" in November, and "B" in December, 1950. This was considered advisable to avoid depleting the colony. The three collections when taken together actually constituted a single collection for the three months, and as carried out kept the entire colony under monthly observations.

Shimosanjo - In this vicinity the colony under consideration was identical with the one studied previously. Immature infections began appearing in this colony in September, 1949, and comprised about 40% of the total infections during the winter and spring months, including June. The adult snail population was greatly reduced in June and July, but there was evidence of extensive propagation. The incidence of snail infections with S. japonicum dropped markedly in July due to the development of numbers of uninfected

progeny. However, mature snails retained an incidence comparable to that of preceding months. If it were not possible to make this distinction it might appear that infections of individual snails had been exhausted.

Aburakawa - The colony sampled in 1950 differs in location from that followed from 1947 to 1949. The snails and the infections were both mature during the winter of 1949-1950. The population was depleted in June, but repopulated by progeny in July only to be depleted again in September.

Shida - The colony here contributed no data of importance, since the infection rate was nil and an inadvertent application of "lime nitrogen" by a farmer destroyed the colony.

Propagation, Growth, and Life Span of the Snail - At Shimosanjo and Aburakawa (Figs. 2, 3) the snail progeny first appeared in numbers at the time of the July collection. At Mutsusawa (Fig. 4) young snails appeared in June and more young were found in July. This was typical only of subdivision "A" of the colony for at "B", and "C" relatively few appeared throughout the entire summer. Based on previous observations (4) the young snails in the June collection at Mutsusawa must have been hatched early in May and the eggs layed during the last half of April.

Growth to four mm. in the sixth week after hatching was noted in snails studied by Sugiura (4). McMullen (8) secured essentially similar results in finding an average growth of 1.5 mm. per month from July to October. Current data from Mutsusawa colony subdivision "A" indicate that growth in 1950 began as early as March and that increase in size of approximately three mm. took place in about four and a half weeks between July and August (Fig. 4), thus again substantiating the earlier findings.

In the eight to nine weeks (July - September) between collections at Shimosanjo and Aburakawa (Figs. 2,3) a growth of three mm. occurred. Adult snails were almost depleted in these colonies by July with the result that the comparisons are based chiefly on the 1950 progeny.

Considerable doubt exists as to the life span of Q. nosophora in the Kofu area. McMullen (8) observed a colony which persisted despite the absence of reproduction for two years, and Sugiura (4) recovered a few live individuals after a five year life span from a group that was painted and returned to natural conditions. There is some evidence that suggests these snails rarely live so long. As shown above, snails which hatch in early summer reach a mode of at least seven mm. by the time of hibernation in the first fall. Sugiura (4) has indicated that a new generation reaches 6.5 to 9.0 mm. in length by the end of the first summer, so that with some growth occurring the following spring they approach the maximum size of nine mm. within a year. If the life expectancy is more than one year, there should be a marked accumulation of eight mm. to nine mm. snails. This, however, is not true, as the growth curves show (Figs. 2, 3, 4). Further, there is evidence of a high mortality among eight mm. to nine mm. snails between the June and July collections at all three colonies. This was also evident in May at Mutsusawa, which was the only colony where young were found in June.

Thus it is apparent that there is a marked mortality among snails reaching one year of age. It has been demonstrated that some do live longer than a year (4, 8). This is supported by our own observation of infections persisting for such a period that the snails bearing them must have been at least eighteen months old and included a second period of hibernation.

Infectivity of Young and Mature Snails - McMullen (9) in 1948 did not find a significant difference in the infectivity of different aged snails at Aburakawa station. However, data from the current Mutsusawa collecting station from December, 1949 to June, 1950, show a significantly higher incidence of infection in young snails (Table XI). 3.7% of the latter were infected compared with 2.2% of the adults, which is a significant difference on the basis of the Chi-squared test (10). Another observation tends to increase this difference. Basic data show that snails of this colony measuring only 3.5 mm. or less in length were not infected. This probably means that they were not in existence at the time of exposure, unless of course it should be shown that very young snails were not susceptible. This would be unusual as most hosts tend to develop an age immunity as they grow older. If these were excluded the above differential would be even greater.

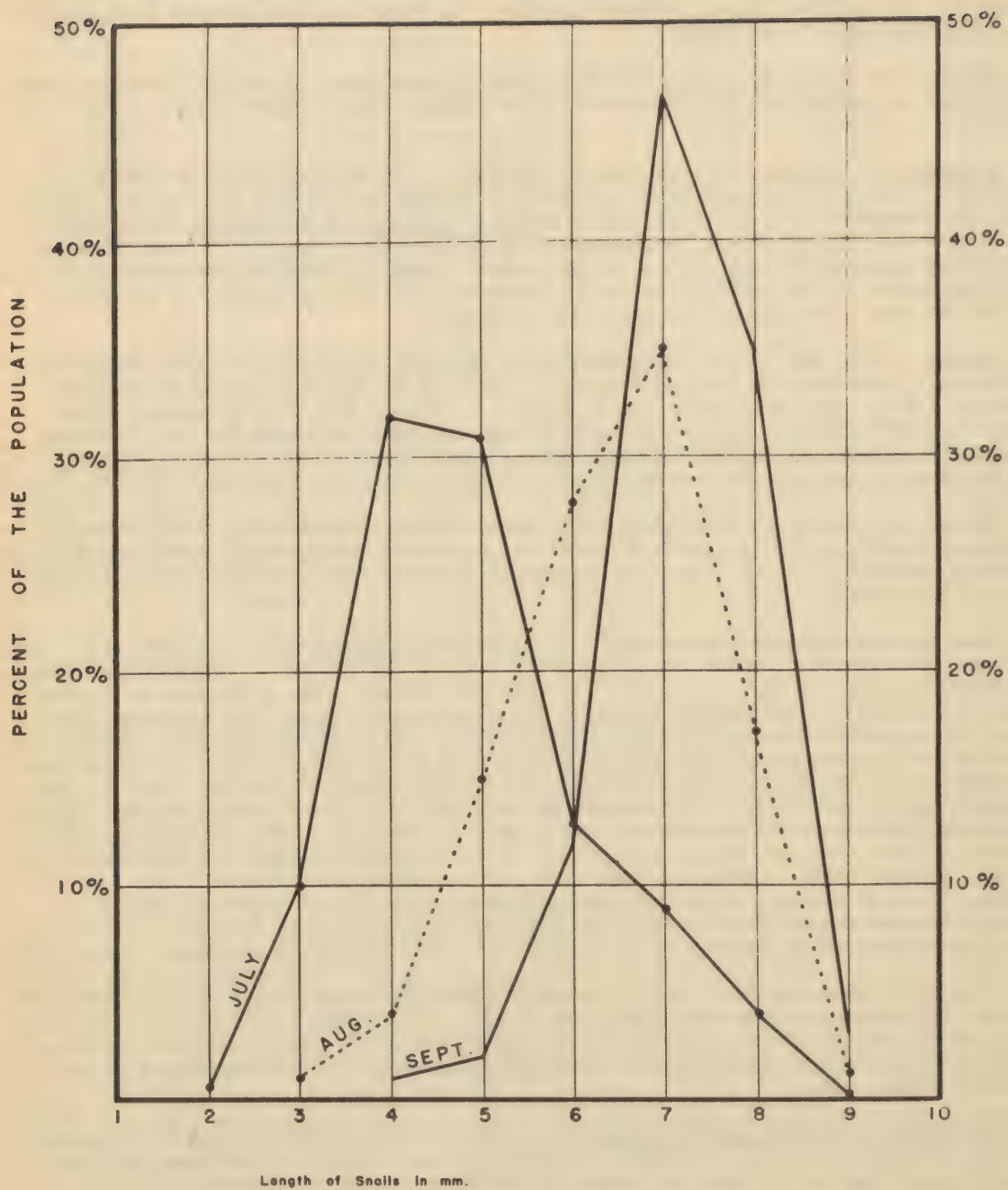
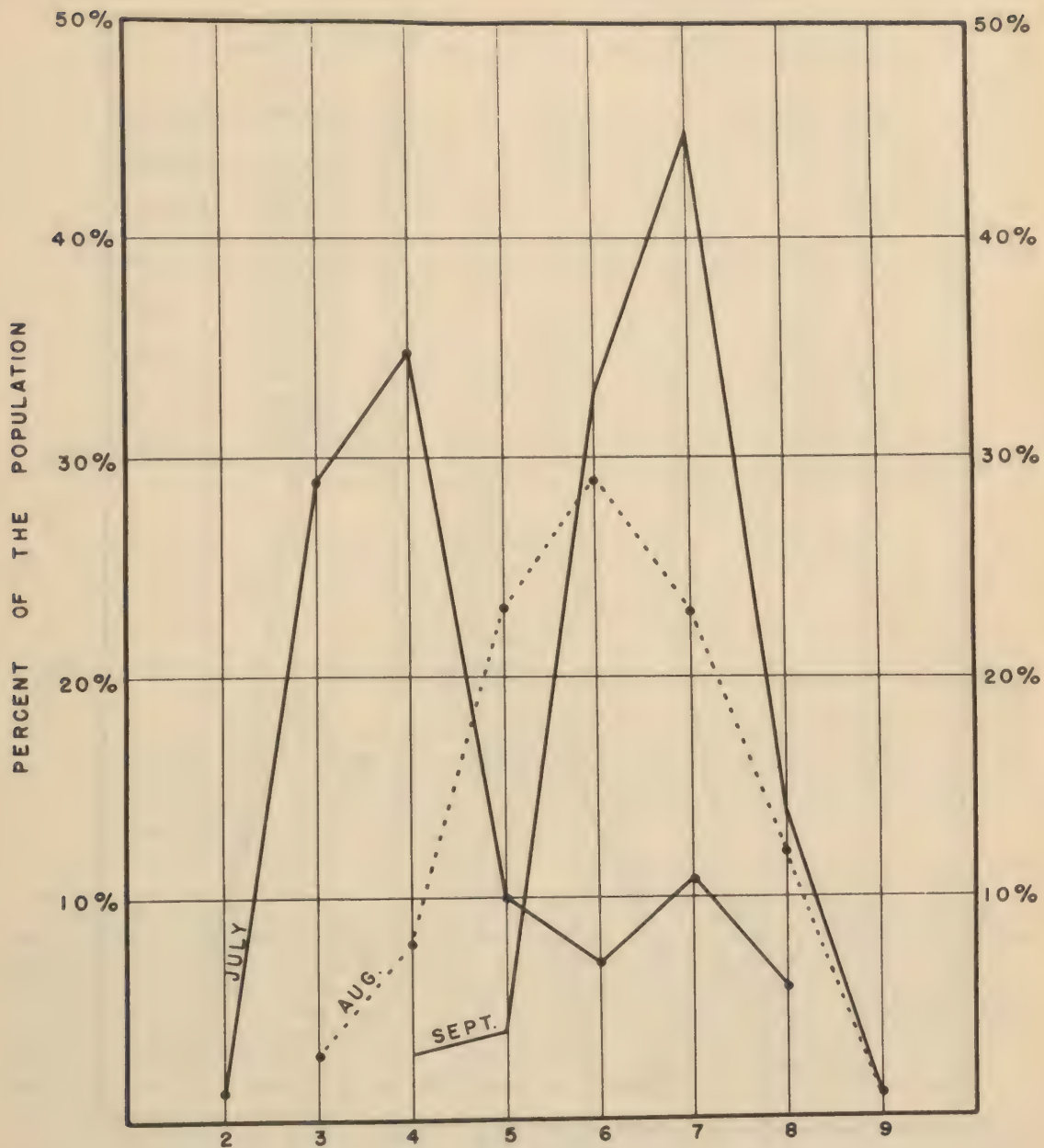


FIGURE 2
GROWTH OF ONCOMELANIA NOSOPHORA
(SHIMOSANJO, YAMANASHI, 1950)



Length of Snails in mm.

FIGURE 3
GROWTH OF *ONCOMELANIA NOSOPHORA*
(ABURAKAWA, YAMANASHI, 1950)

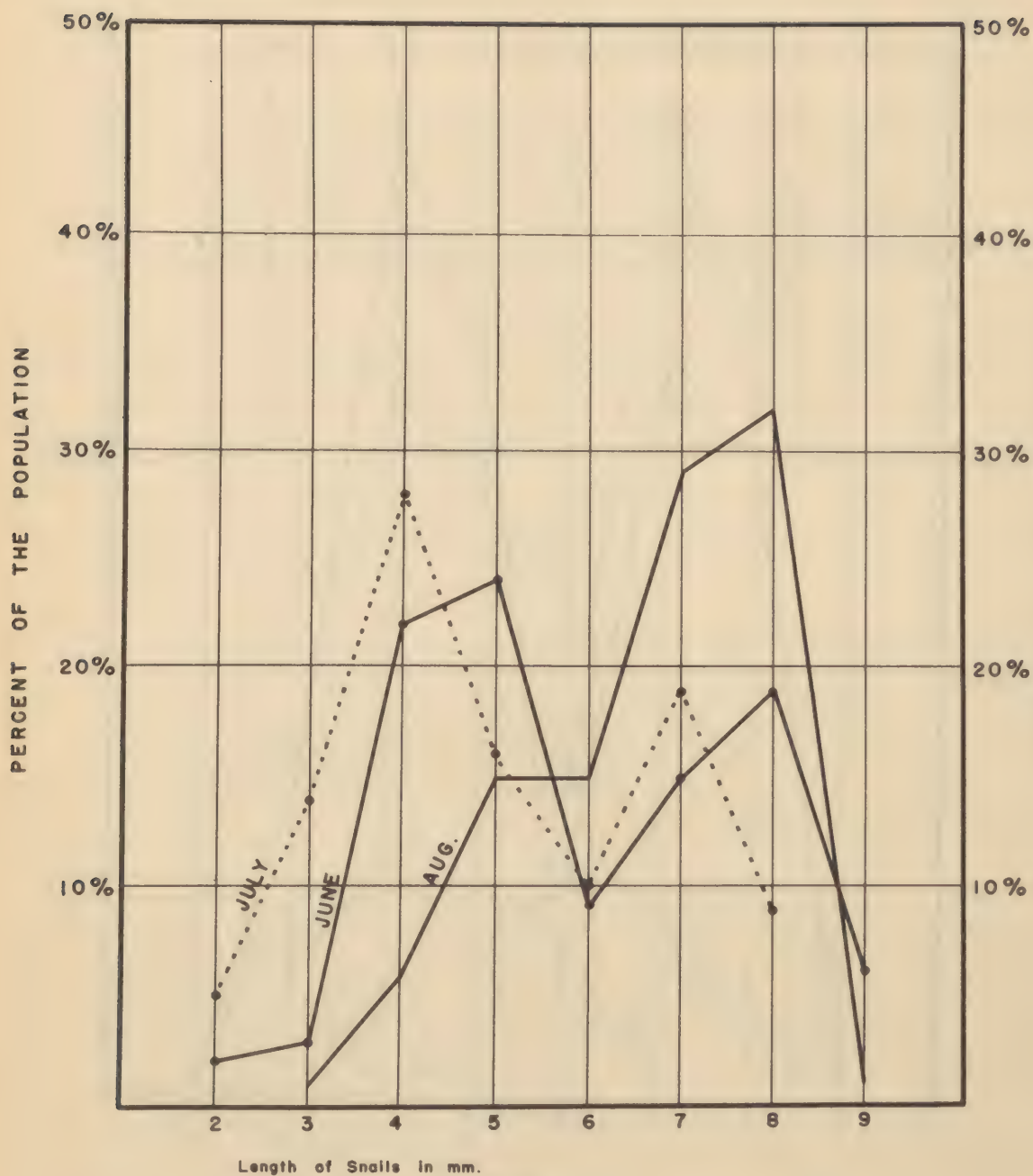


FIGURE 4

GROWTH OF *ONCOMELANIA NOSOPHORA*
 (MUTSUSAWA "A" STATION, YAMANASHI, 1950)

Table XI. Comparison of Infections in Young and Mature Snails (Mutsusawa)
December, 1949 - June, 1950

			No. and Incidence of Infections of Varying Ages*		
Maturity and No. of Snails			a-d	e-g	h-i
<u>December</u>	Young	193	5	0	0
	Mature	314	5	2	7
<u>January</u>	Young	214	8	0	0
	Mature	314	9	4	14
<u>February</u>	Young	283	7	0	0
	Mature	224	4	3	5
<u>March</u>	Young	206	5	2	0
	Mature	442	5	8	9
<u>April</u>	Young	111	7	1	0
	Mature	480	10	6	35
<u>May</u>	Young	59	5	2	0
	Mature	369	11	3	16
<u>June</u>	Young	159	8	1	0
	Mature	333	11	9	9
<u>Total</u>	Young	1225	45	6	0
	Mature	2476	55	35	95

* a - 2 weeks
b - 3 weeks
c - 4-5 weeks
d - 6 weeks
e - 7 weeks
f - 8 weeks
g - 10 weeks
h - 20 weeks
i - 40 weeks

These ages are based on the appearance of the infection and the time required for development to reach the stage present under optimum conditions. Immature includes up to 8 weeks, young mature up to 10 weeks and mature infections include all others. (Adapted from Faust and Meleney (1)).

In this comparison of infectivity only infections of ages a - d inclusive were used. Older infections were ruled out because they very probably occurred before the young snails of the colony were hatched; the data indicate as much for ages e - g. Mature infections are rarely found in young snails as snails tend to reach maturity in a shorter period of time than does the infection which they bear. McMullen's comparison was based on young infections of all ages. Further, it might be noted that an influx of young snails occurred at Aburakawa in July, 1949 for which there may have been less opportunity of exposure than for pre-existing old and young snails.

As many young infections existed throughout the winter and spring months at Aburakawa, the data from October, 1947 through June, 1948 might have given a more adequate evaluation of the relative infectivity of young and mature snails.

There is evidence, then, that snails 4-5.5 mm. inclusive are more apt to become infected than those 6 mm. and over. This difference may be due to opportunity of exposure rather than susceptibility, as young snails have been noted to be in water or at its edge more than mature ones, and presumably the miracidium can enter the snail only when the latter is in the water.

The Incidence of *Schistosoma japonicum* in *Oncomelania nosophora* - The occurrence of snail infections varies greatly for, and within, the several endemic centers of schistosomiasis in Japan. For a circumscribed collecting area the infection status changes over a period of months. The current data further show that even within very limited areas the snail population is not uniformly infected. As it is difficult to collect uniformly over the circumscribed area each month inconsistencies appear in the findings which may lead to erroneous conclusions; this will be emphasized subsequently.

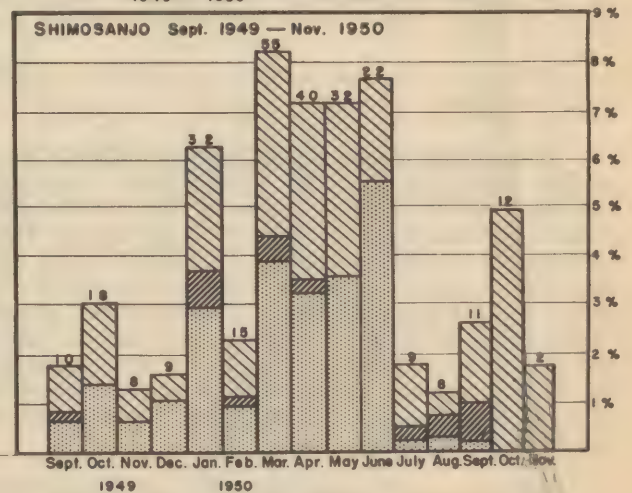
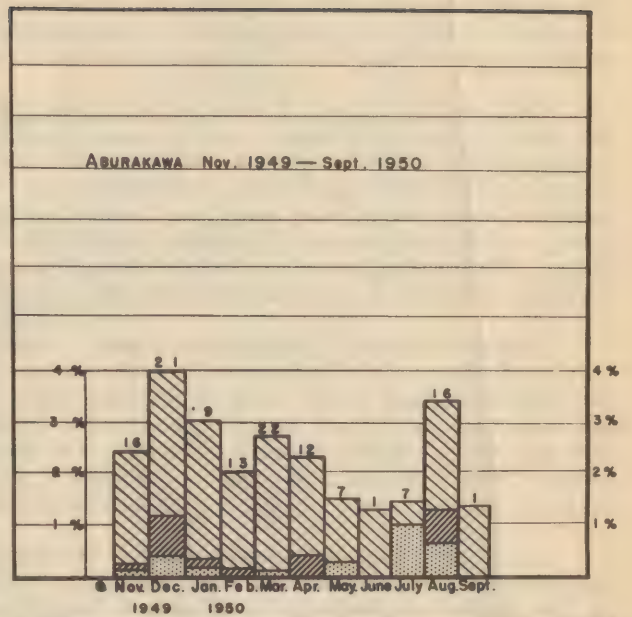
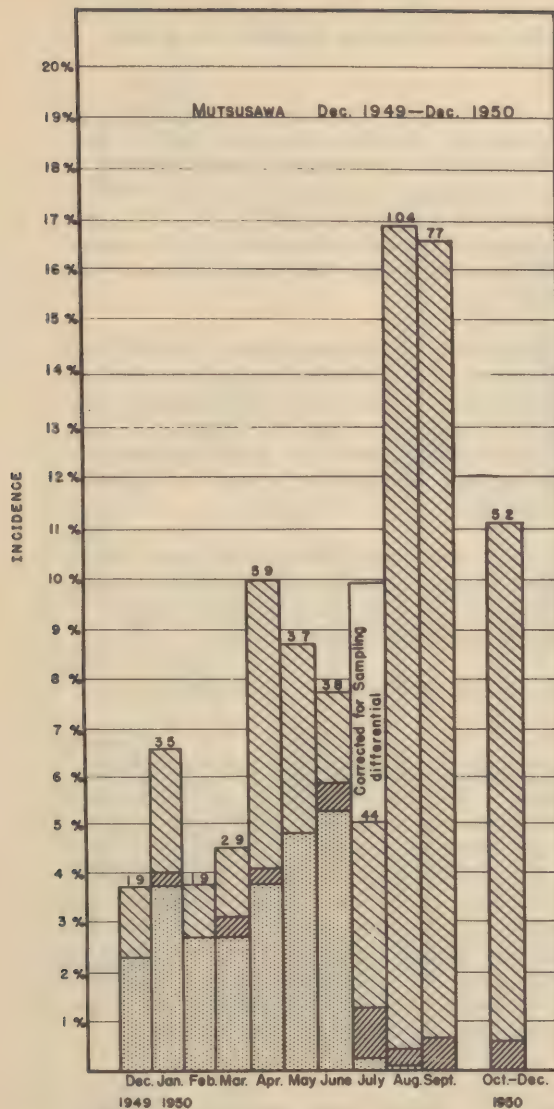
Time of Occurrence, Maturation and Duration of Infections - McMullen's data (9) indicate clearly the likelihood of young infections appearing in July collections. It occurred in both years at Aburakawa and once each at Mutsusawa and Shida. At Shimosanjo young infections occurred in May. Exposures at other times were suggested, but the evidence was not conclusive since either the increase did not persist in subsequent months or it was based on an increase of only mature infections.

During the winter and spring of 1949-50 the collections at Shimosanjo contained immature infections to the extent of about 50% (Fig. 5). On tracing these back, it was noted that they appeared first in the September collection. Immature infections were even more predominant in the Mutsusawa collections during the winter of 1949-50, but their history could not be traced. However, it will be noted in a previous section (Table XI) that the infections did not occur in snails under 4 mm. in length. This suggests that these young snails were not in the colony at the time of exposure (unless, of course, very young snails are not susceptible). The very young uninfected snails must have been hatched in late August or early September. The exposure accounting for the young infections at Mutsusawa, then, probably did not occur later than early September which coincides with the above observations for Shimosanjo.

Regarding the maturation of infections, one point is clear. They commonly pass hibernation in the immature state (9) and remain essentially static until June or later, attaining maturity in July (Fig. 5). Data previously collected (9) show that some young infections appearing in July mature only to the young mature stage by the time of hibernation; others apparently reach maturity. May infections (Shimosanjo 1949) had reached maturity by August.

Regarding the duration of infection, additional information is furnished by the Mutsusawa colony currently under observation. Young infections contracted presumably in late summer 1949, matured in July 1950. For the remainder of the summer the infections were mostly mature, and the incidence did not decline as predicted (9). It has been shown that the apparent drop in the July incidence at Mutsusawa (Fig. 5) was not a real one. The identity of the snails from several substations ("A", "B", and "C") was maintained and it was noted that subcolony "A" had an incidence of only about one-fifth that of "B" and "C". A critical check of data revealed that an excess of the snails came from substation "A" that month, thus accounting for the apparent drop in incidence. When mathematical correction was made for this sampling differential, the incidence for July falls just short of 10% and coincides with preceding and subsequent months. The question then arises whether any of the infections originated during the spring or early summer of 1950, as McMullen contends that infections carried through the winter are exhausted during the summer and the incidence declines unless spring or summer infections occur. Although a categorical statement can not be made, there are indications that the sustained infection rate for the late summer and fall months of 1950 include many of the infections carried over from the previous year. For the Mutsusawa colony as a whole there was some increase, beginning in April, of infections of the ages a - d (Table XI). However, from an examination of basic data it becomes evident that they were quite inadequate to account for all the infections persisting as mature ones during the late summer and fall of 1950.

Summary - Young snails appeared in the June as well as the July collections of one station. A growth of 3 mm. occurred between July and August collections of one colony, and a 3 mm. growth from July to September was observed for two others. It appears that snails often attain maximum growth in one year. Although they are believed to live for two to five years, evidence is presented which indicates that the usual life-span is only one year: (i) higher mortality was apparent in the largest size categories in July, and in one instance in May; (ii) and further there is no tendency for an accumulation of the larger snails, which should be the case if they reach the maximum size in one year and live for several years. It was shown that young snails 4-5.5 mm. inclusive are more apt to be infected than those larger. Current data



32 TOTAL INFECTIONS
 MATURE INFECTIONS
 YOUNG MATURE
 IMMATURE

FIGURE 5

MONTHLY FINDINGS ON INCIDENCE AND MATURITY OF *S. JAPONICUM* INFECTIONS IN *O. NOSOPHORA*, YAMANASHI PREFECTURE, 1950

indicate that variations of infections occur within very small areas and may account for irregularities in the monthly data which stimulates erroneous conclusions. The fact of infections occurring in late summer as well as in July (the time most commonly observed) is substantiated. The fact of immature infections persisting through the fall and winter months and remaining as such until the following July is emphasized. Evidence is offered which suggests that such infections reaching maturity in July are not necessarily exhausted during the subsequent summer months, but may persist through a second winter. The possibility of July infections not maturing beyond the young-mature stage by the time of hibernation was also noted.

FIELD PLOT TESTS OF POTENTIAL MOLLUSCACIDES: Because of certain discrepancies in the data secured previously, and because freshly prepared samples were available, the molluscicidal activity of 50% chlordane and 25% heptachlor in wettable powder were tested in the field near Kofu, Yamanashi-ken. Two adjoining irrigation ditches were selected, each containing many snails and having similar physical conditions such as depth, width, and amount of vegetation. Each ditch was divided into four 25 ft. sections. The control consisted of a similar segment located above the test ditches, thus avoiding downstream contamination by the chemicals.

In each section, pre-treatment snail counts were made in three equally spaced one foot quadrats and the per cent of dead snails determined. The same dosage was used for both chemicals which were applied by means of sprinkler cans after the vegetation was removed. Each test section received a different dosage of chemical dissolved in five gallons of water, namely 50 grams, 100 grams, 200 grams, and 500 grams respectively. Four and eight days after treatment, snail counts were made in one foot quadrats adjacent to those used for the pre-treatment counts.

Using chlordane 3.9% and 13.8% of the snails were dead four and eight days respectively after treatment as compared with 3.0% dead in the pre-treatment collections. In the case of heptachlor, 2.0% of the snails were dead in the pre-treatment count; four and eight days after treatment 5.0% and 10.0% respectively were dead.

The above results indicate that these substances as used were not satisfactory molluscicides.

THE DISTRIBUTION OF ONCOMELANIA NOSOPHORA, THE SNAIL INTERMEDIATE HOST OF SCHISTOSOMA JAPONICUM ALONG THE TONE RIVER: Introduction - The actual status of schistosomiasis and the distribution of the snail intermediate host, *Oncomelania nosophora*, along the Tone river in east-central Honshu have not been adequately determined. According to the investigation of Wright et al (5) the entire Tone valley at least from Sakai to Sawara should be considered a suspected endemic area, with foci at Sakura and Sawara, although they were not themselves successful in locating snails. Japanese investigators in 1914-15 noted both human infections and snail colonies on both sides of the river in the areas adjacent to the opposing villages of Toride and Abiko, which are midway between Sawara and Sakai. Olivier (6) investigated the status of the disease at Koya, several miles above Toride, and mapped the location of the snails in the vicinity (Figs. 6, 7).

A survey of *O. nosophora* was initiated in the fall of 1948 and completed in May of 1950. Both sides of the river were systematically searched for snails from Sakai to Sawara, a distance of approximately 50 miles. Also endemic foci at Sakura, Chiba Prefecture, and in the Towa-Hikonari area, Saitama Prefecture, were investigated.

Methods - It had been previously recognized and currently confirmed that the snails are almost entirely limited to the extensive marshland between the levees or natural embankments and the river channel. Although favorable habitats outside the levees were searched, attention was chiefly given to the former areas. Commonly a search was made at about half mile intervals just inside the levees. A critical selection of points for searching was made possible by recognizing certain favorable conditions of habitat, which included: (i) considerable width between the levee and the main channel, (ii) recesses and sharply-defined coves in dykes or natural embankments serving as protection against flood currents, (iii) heavy vegetation such as marsh grass or reeds, scrub trees, willow or rosebush clumps, and (iv) dampness of soil

resulting from dew or light rains and maintained by denseness of vegetation. At Sakura and in the Towa-Hikonari vicinity the search for snails was determined by epidemiologic data previously collected or information furnished by local sources.

Findings - The following findings include data collected both in the fall of 1948 and the spring of 1950 (Table XII). Multiple points of search were made at many of the places designated in the table. Twenty-seven places were searched on the Chiba side of the river, and snails were located at 13 of these (Figs. 6, 7). Collections were relatively light except in two places. In a marshy meadow area at Tanaka, well up river, 767 snails were located, none of which were infected. Just above Sawara at Kawajiri 226 snails were collected during May 1950, at a point where the dense marsh grass had recently been cut. None of these were infected. The above colonies had been centers of propagation the previous season, as both included some young snails. Of all the collections on the Chiba Prefecture side of the river, positive snails were found only at Kobuke, where two of a collection of 15 were infected.

On the north side of the river in Ibaraki Prefecture, 25 places were searched. Snails were located at 17 of these, and the collections for five included over 100 snails each. Four of these sizeable colonies were in the middle third of that portion of the river investigated between Ono and Toride; the other was at Sawara, the farthest point studied downstream. The two colonies at Koya have been observed for four years (1946-1950); they have persisted in spite of numerous inundations, several of which covered the area to a depth of over ten feet. Whereas 407 snails were collected at Ono with ease in 1948, only a few could be found in 1950. Floods had carried in considerable deposits of sand. The colony at Toride has persisted for two years. The one at Sawara has been observed only once, but included young snails (1950).

Infected snails were found on the Ibaraki side only in the two Koya colonies and nearby Togashiro (1948). Thus for the entire survey, infected snails occurred in four of thirty collections.

Although an intensive search was made at both Sakura and in the Towa-Hikonari area, snails were not found in either place.

Discussion - The current study indicates that a respectable snail population exists from the junction of the Kinu river downstream as far as Sawara, a distance of approximately 40 miles. Although a few populous colonies were encountered, most of the area was sparsely populated. Only the Fusa-Fukawa area of this sector of the river is obviously unsuitable for snails. The small size of most of the collections does not mean necessarily that the snail population is inconsequential. First, some dense colonies could easily have been overlooked, and secondly, a few snails in a unit area could mean great numbers for the entire river bed. Snails are widely scattered over the marshlands along the Tone river whereas in many other endemic areas in Japan they are localized in irrigation ditches. The probable reason why the Tone valley is not a serious endemic center is the fact that the people go only infrequently into the areas where the snails are located. In contrast to other endemic centers where the snails show a predilection for the farming conditions associated with rice culture, they appear to have receded in the Tone valley with the building of levees which in turn made possible increased rice culture.

It would appear that snails are more numerous on the Ibaraki Prefecture side of the river. The search for them was more frequently successful on this side, and sizeable colonies were more often encountered.

Our collections extended well beyond the limits of the endemic focus adjacent to Sawara recognized by Wright et al (1947)(5) These authors did not locate snails, their report being based on information furnished by Japanese health officials and a limited number of stool examinations. The report of a snail colony by Olivier at Koya is the only one of its kind in recent years. His review of Japanese literature indicates areas where Japanese investigators located snails in 1914-15. In these same general areas snail populations still exist after 35 years.

Table XII. Localities Examined for Oncomelania nosophora (1948-1950), the Intermediate Host of Schistosoma japonicum

CHIBA PREFECTURE (From Sakai downriver)				IBARAKI PREFECTURE (From Sakai downriver)			
Locality	No. of Snails			Locality	No. of Snails		
	Total	Mature	Immature		Total	Mature	Immature
SEKIYADO-MACHI	0			NAGASU-MURA			
SHIMONOYA	0			Komagase	0		
KAWANA-MURA	0			NANAGO-MURA			
ASAHI-MURA	8	8	0	Yahagi	0		
FUKUDA-MURA	0			ONO-MURA	407	**	**
TANAKA-MURA	767	734	33	*KOYA-MURA (a)	107	**	**
TOMISEI-MURA	0			*KOYA-MURA (b)	848	**	**
Kujiya	1	1	0	INADOI-MURA			
ABIKO-MACHI				* Togashira	81	**	**
Shibasaki	22	22	0	Inadoi	17	16	1
Aoyama (a)	7	7	0	Ina	27	22	5
Aoyama (b)	2	2	0	TORIDE-MACHI (a)	81	71	10
KOHOKU-MURA				TORIDE-MACHI (b)	366	319	47
Kobori	0			Yoshida	16	15	1
Furuto	0			OMOMMA-MURA			
FUSA-MACHI				Nakatsuma	0		
Ezochi	0			Minami	1	1	0
OMORI-MACHI	0			Todai	32	19	13
FUKAMA-MURA				FUMI-MURA			
Fuda	0			Kamisone	0		
Nakaya	0			RYUGASAKI-MACHI			
Idetsu	2	2	0	Kyugasakichobu	0		
TOYOZUMI-MURA				MANAITA-MURA			
Yaguchi	0			Daitoku (a)	13	13	0
Tatsudai	15	15	0	Daitoku (b)	11	11	0
TAKAOKA-MURA				KANAEZU-MURA			
Takaoka	1	1	0	Kanaezu	0		
*Kobuke	15	14	1	TOYOSHIMA-MURA			
KOZAKI-MACHI				Yotsuya (a)	0		
Kozaki	10	10	0	Yotsuya (b)	17	17	0
Ima	0			Oshisuma	0		
HIGASHIOTO-MURA				Rokkaku	4	4	0
Kawajiri	226	132	94	SAWARA-MACHI (a)	162	154	8
Tatajima	6	6	0	SAWARA-MACHI (b)	38	32	6
SAWARA-MACHI	0						
1082				2228			

/ Aged shells found in loose soil on the bank of a small lake immediately outside the levee

* Infected Snails found

** No measurement made

Summary - The 50 mile sector of the Tone river from Sakai to Sawara was searched on both sides of the channel for O. nosophora, the snail intermediate host of S. japonicum. As far down stream as the Kinu river junction snails were not commonly found. From this point to Sawara, a distance of about 35 miles, they were frequently found on both sides of the river. It appears that colonies are found more commonly and almost uninterruptedly on the northern, or Ibaraki side of the river for a distance of about ten miles between the Kinu and Kogai river junctions. Of all the snail collections, infected snails were found in only four.

STUDIES ON THE DISTRIBUTION OF ONCOMELANIA NOSOPHORA, THE SNAIL INTERMEDIATE HOST OF SCHISTOSOMA JAPONICUM ON KYUSHU: In the area surrounding Tosu in the eastern edge of Saga, and Kurume in the south western portion of Fukuoka Prefecture, lies one of the largest endemic areas of schistosomiasis in Japan (Fig. 8). Members of the Commission on Schistosomiasis who briefly visited this area in 1945 (5) verified the continued endemicity of this disease. Subsequently in 1948 it was investigated more thoroughly when a joint epidemiologic survey was undertaken with the Japanese National Institute of Health (1, 7). At that time this area was found to be one of the most heavily parasitized schistosomiasis regions that had been encountered in Japan. Both the incidence and parasite density index were higher than had been reported from any other known endemic center (1).

The snail survey in 1948 was made in conjunction with the general epidemiologic survey of Kyushu mentioned above. At this time the study was limited to the areas near the heavy schistosomiasis centers of Tosu-machi and Kurume-shi in Saga and Fukuoka Prefectures respectively. Irrigation ditches, streams, and rice paddies were thoroughly examined for O. nosophora. The viable snails collected were crushed and the percentages of infection with S. japonicum determined (Table XIII).

A total of 26 villages in 16 townships were surveyed (Table XIV); in 7 townships 2634 snails altogether were found, of which 130, or 4.9%, were infected with S. japonicum (Table XIII).

During 1950 a more intensive survey was instituted in an attempt to more clearly circumscribe the endemic area of schistosomiasis. This survey was limited almost entirely to Fukuoka Prefecture, since it comprises the greater portion of the endemic area. The survey began in the vicinity of Kurume City and extended northeast, passing beyond the boundary of the area regarded as endemic by the Schistosomiasis Commission in 1945 (5). A few areas were examined southwest of Kurume in Saga Prefecture. Thirty-seven settlements located in 16 different townships were surveyed and snails were found near 20 of these small villages (Table XIV). A total of 1754 snails were crushed, of which 49, or 2.8%, were infected with S. japonicum (Table XIII).

Infected snails were found in three townships outside the known endemic area, thereby extending the known limits of schistosomiasis in this endemic region (Fig. 9)

Summary - The survey revealed that the distribution and population centers of the snails were not uniform throughout the endemic area and that the snails were not equally infected in the various communities. It was found that the endemic area covers a larger area than was previously recognized and that additional, more intensive surveys are needed to clearly circumscribe the region.

LABORATORY STUDIES OF SCHISTOSOMA JAPONICUM INFECTIONS IN THE INTERMEDIATE SNAIL HOST ONCOMELANIA NOSOPHORA: Introduction - A series of laboratory experiments were initiated in 1947 in an attempt to secure fundamental data on various aspects of the life cycle of Schistosoma japonicum. These included: (i) the general and daily cercarial shedding pattern in both laboratory and naturally infected snails; (ii) the influence of such factors as light, temperature, and pH on shedding of laboratory and naturally infected snails; (iii) the effects of temperature and fecal dilution on egg viability.

Experimental attempts to infect O. nosophora - Beginning in 1947 snails were exposed to miracidia in different ways in an attempt to secure laboratory infections (1, 3). In July, 1949 snails were exposed individually to the miracidia of S. japonicum. An attempt was made to determine (i) the infectivity of the miracidia for both young and mature snails, (ii) the infectivity of miracidia from both cow and human sources, and (iii) the resulting incidence of infection from exposure to one and two miracidia. Exposures were made by placing the snail and miracidium in a few drops of water, the snail being forcibly kept in the water for 30 minutes.

Table XIII. Incidence of Schistosoma japonicum in Oncomelania nosophora,
Collected from the Endemic Area of Schistosomiasis in Kyushu,
1948-1950

FUKUOKA PREFECTURE						
	1948			1950		
	No. of Snails Collected	No. Infect- ed with <u>S.</u> japonicum	Per cent Infected	No. of Snails Collected	No. infect- ed with <u>S.</u> japonicum	Per cent Infected
AMAGI-MACHI						
Hitotsugi				11	0	
MADA-MURA						
Kamiura-kami				161	0	
Kamiura-shimo				100	0	
MIHARA-MURA						
FUKUDA-MURA	3	0				
Hirazuka				19	1	5.3
AJISAKA-MURA	75	0				
Yasaka				61	0	
Tsukuride				73	1	1.4
KITANO-MACHI						
Juromaru				180	0	
Kogachaya				130	0	
MIYANOJIN-MURA						
Inabuchi				66	1	1.5
Tomiyasu				100		
KURUME-SHI						
Nagatoishi-cho	938	68	7.2	853	46	5.4
Total	1016	68	6.7	1754	49	2.8
SAGA PREFECTURE						
TASHIRO-MACHI	38	0	-			
TOSU-MACHI						
Anrakuji	979	53	5.4			
ASAHI-MURA	601	9	1.5			
Total	1618	62	3.8			
Grand Total	2634	130	4.9	1754	49	2.8

The data (Table XV) suggest that young snails are more susceptible to infection than old ones; 15.4% of 39 young snails became infected and only 4.7% of 107 mature ones. This difference is fairly significant as determined by the Chi-squared test (10). A similar observation has been made from field studies in the above section (Seasonal Studies). A considerable difference was noted for the infectivity of miracidia obtained from human and cow stools. An incidence of 4.7% was obtained for snails exposed to a single miracidium from the former source and 23.5% when the miracidium was obtained from a cow stool. Two miracidia did not account for a significantly higher incidence than a single one. Whether or not this is significant or whether only one miracidium penetrated and survived cannot be ascertained at present.

Table XIV. Summary of Findings and Areas Surveyed for Schistosomiasis
Bearing Snails in Kyushu (1948-1950)

FUKUOKA PREFECTURE					
Township	Results of Survey		Township	Results of Survey	
	1948	1950		1948	1950
YORII-MACHI			KANESHIMA-MURA		
Ishihara		/	Yaegame		0
Iyanaga		0	OKI-MURA		
ALAGI-MACHI			Oki	0	
Hitotsugi		-	Otoyoshi		0
Chiyomaru		-	Tsukajima		0
MADA-MURA			MIYANOJIN-MURA		
Mada	/		Tomiyasu		-
Kamiurakami		-	Inabuchi		/
Kamiurashimo		-	Goromaru		/
Honkamiura		/	KITANO-MACHI		
TACHIARA-MURA			Juromaru		-
Matsuzaki	0		Kogachaya		-
Yamakuma	0		Jinya		0
Ushikawa		0	Nakamura		0
Unoki		0	YUGE-MURA		
Imamura		0	Haizuka		-
FUKUDA-MURA			AIKAWA-MURA		
Hirazuka		/	Edamitsu		0
Inakazu		0	KURUME-SHI		
OGORI-MACHI			Nagatoishi	/	/
Ogori	0		Sasayama	/	
AJISAKA-MURA			Higashikushihara		/
Kaminishiajisaka	0		Ichinokami		/
Hatama	0		ZENDOJI		
Juraku	-		Zendoji	0	
Yasaka		-	TSUBUKUIMA-CHO		
Tsukuride		/	Tsubukuima	0	
Ashima		0	AKAKI-MURA		
Ohashi		0	Shirakuchi	0	
Kanatsubo		0			
MIHARA-MURA					
Shimotakahashi	-				
HONGO-MACHI					
Koga		0			
Kojo		0			
SAGA PREFECTURE					
KIYALA-MACHI			TASHIRO-MACHI		
Kubota	0		Ikeda	0	
Nagano	0		Hatazaki	0	
Noguchi	0		KISATO-MURA		
TOSU-MACHI			Sonezaki	-	
Anrakuji	/		ASAHI-MURA		
Todoroki	-		Gitoku	-	
MAKEZU-MURA			Yasura	-	
Makezu		/	Okusu	/	
KITASHIGEYASU-MURA			MINAMISHIGEYASU-MURA		
Nakano		0	Doisoto	0	
Ekuchi		-			
Higashibun		0			

Legend - Snails found but not infected
/ Snails found and infected
0 Snails not found



FIGURE 8 — MAP OF KYUSHU SHOWING AREA
ENDEMIC FOR SCHISTOSOMIASIS JAPONICA.



FIGURE 9— DETAILED MAP OF AREA ENDEMIC FOR SCHISTOSOMIASIS JAPONICA AND LOCALITIES SURVEYED FOR SNAILS.

Table XV. Experimental Infections of Schistosoma japonicum in the Snail Intermediate Host Oncomelania nosophora During 1950

Experimental Conditions				Result		
No. of Snails Exposed	Age of Snail	Source of Eggs	No. of Miracidia Used	No. Snails Surviving	No. Infected	% Infected
132	Mature	Human	1	107	5	4.7
54	Young	Human	1	39	6	15.4
70	Mature	Cow	1	51	12	23.5
50	Mature	Cow	2	40	11	27.5
Total				237	34	14.3

The Daily Shedding Pattern of Cercariae of S. japonicum from O. nosophora - Naturally infected snails were set up in sputum cups and examined at two hour intervals from 0800 to 2000 and again the following morning at 0800. This procedure was continued over a period of several days. Repeated experiments indicated that shedding occurs chiefly in the hours between 1200 and 0800, with the peak between 1400 and 1600 (Fig. 10). Some shedding did occur after 2000 and a number of variations occurred in the several experiments. Cram (11) observed maximum shedding for O. nosophora as occurring between 1200 and 1600. In contrast, it has been shown by Bauman et al (12) that maximum shedding for O. quadrasi occurs between 2100 and 2300 hours.

The General Shedding Pattern and the Number of Cercariae Shed by Experimentally and Naturally Infected O. nosophora - A shedding experiment, using snails experimentally infected in July 1949, was initiated in December 1949 and continued as long as the snails remained alive. Naturally infected ones were used as controls. Snails were set up for a portion of each day in sputum cups, otherwise they were kept separately on filter paper in petri dishes. Some continued to shed for as long as 140 days. The average number of cercariae shed by each snail for ten day periods is graphically shown in Figure 11 for both experimental and control snails. The initial burst of shedding typical of many infected specimens brought in from nature did not occur for the experimentally infected ones. Probably the infections of the latter were relatively immature at the time the experiment was begun; after 70 days shedding increased. In contrast, very little shedding occurred for the naturally infected snails after 70 days. The average number of cercariae shed by each snail was considerably higher in the case of natural infections (Fig. 11).

Other Factors - The degree of schistosome cercarial shedding by naturally infected Oncomelania nosophora in buffered water from pH 6.0 to 8.0 has been observed in this laboratory. The data (Table XVI) seem to justify the conclusion that the pH within these limits does not affect shedding, though the maximum number of cercariae are probably liberated between pH 6.8 and 7.6. These results are somewhat at variance with those obtained by Bauman et al (12) with the Philippine species, Oncomelania quadrasi, who found a pH 7.6 to be optimum and that a pH below 7.0 completely inhibited shedding.

Table XVI. Effect of pH on the Shedding of Cercariae of Schistosoma japonicum in Oncomelania nosophora in 1950

pH	No. Snails	Total No. Cercariae	Average No. Cercariae
6.0	10	8,690	869.0
6.8	26	24,047	924.9
7.2	18	17,823	990.2
7.6	19	16,541	870.6
8.0	21	11,487	547.0

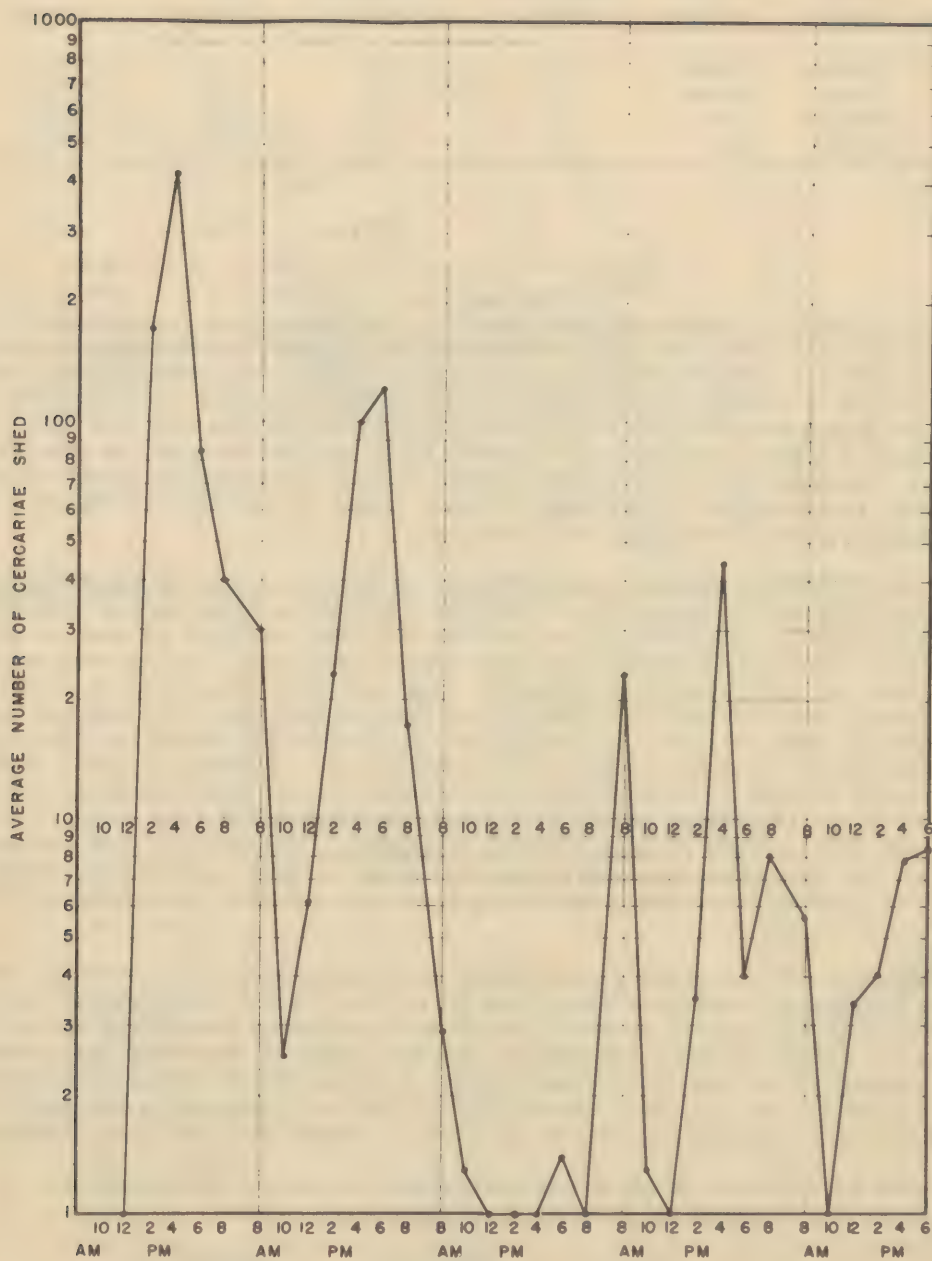
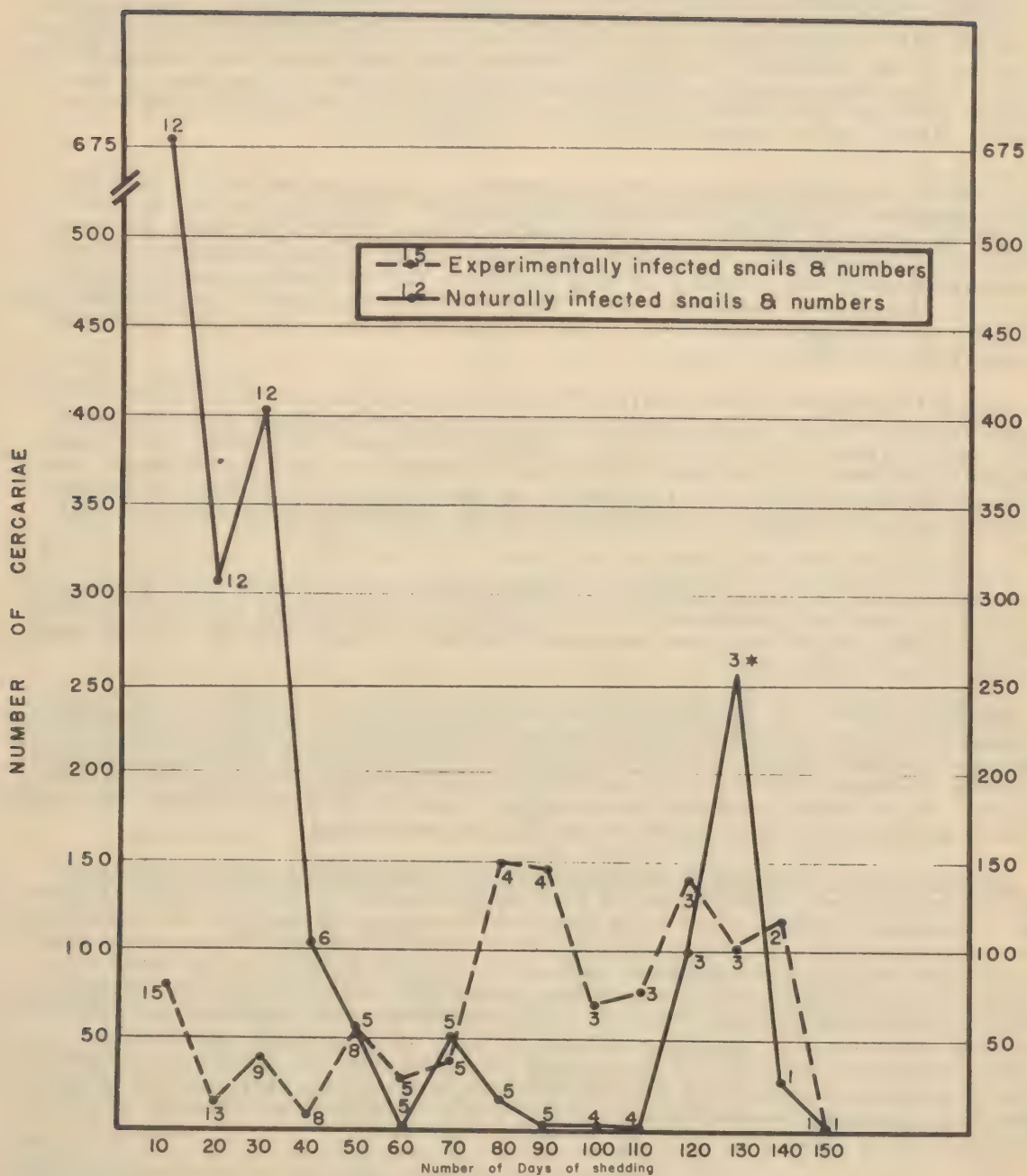


FIGURE 10

AVERAGE DAILY SHEDDING PATTERN OF CERCARIAE OF
SCHISTOSOMA JAPONICUM FROM ONCOMELANIA NOSOPHORA IN
 1950



* This peak was accounted for by a single snail

FIGURE 11
SHEDDING PATTERN OF NATURALLY AND EXPERIMENTALLY
INFECTED ONCOMELANIA NOSOPHORA IN 1950

Results of experiments on the effect of light on shedding of schistosome cercariae from the O. nosophora snail are conflicting. Work on this project is being continued, since the results appear to differ from those obtained by the Philippine group (12).

The Effects of Temperature and Fecal Dilutions on Egg Viability - In order to secure additional data on the factors governing the hatching of Schistosoma japonicum eggs described by Ingalls, Hunter et al (13) in the Philippines a number of experiments were set up in the laboratory. Ingalls et al found that eggs hatched best between 25° and 30°C. if the feces containing the eggs was thoroughly washed and the water in the flasks was kept alkaline (about pH 7.6). The intensity of the illumination, agitation or osmotic stimuli had no effect.

A tapwater dilution of feces containing eggs of S. japonicum was set aside at an ice-box temperature of 8.0°C. and observed over a period of four months. At the end of this time the eggs still hatched and the miracidia appeared to have the vigor of those hatched from a fresh stool. The project was repeated under varying conditions. Feces was maintained undiluted, diluted 1:10 with tap water, 1:10 with urine, and 1:10 with a mixture of equal parts of urine and water. These dilutions were kept at both summer room temperature and refrigeration of 8°C. It had been noted by Faust (14) that eggs hatch at temperatures over 8°C.

At room temperature (approximately 27°C) only the water-diluted feces contained hatchable eggs at the end of five days, and in it eggs ceased to hatch after two weeks. At 8°C. eggs in feces diluted with urine no longer hatched after five days, while those in undiluted and water diluted feces hatched after 2 weeks, but not after 6 weeks. Variations in the results of the two experiments may have been due to exposure of the eggs to summer temperatures prior to refrigeration, or the 1:10 dilution may have been inadequate.

A third project is being conducted in which feces has been diluted 1:10, 1:50, and 1:100 with tap water and maintained at 8°C. At the end of two months eggs still hatched in all dilutions, but at this point more miracidia were recovered from the 1:50 dilution than either of the other two.

Summary - Study of O. nosophora artificially and naturally infected with S. japonicum suggest that the optimum shedding of cercariae in naturally infected snails occurs between 1400 and 1800 hours at a pH of 6.8 to 7.6. The hatching of eggs of S. japonicum was studied in water, water and urine in varying concentrations, and under varying temperatures. Urine seemed to inhibit the hatching of eggs; however feces diluted with water yielded miracidia after being maintained at 8°C. for two months.

IMMUNITY IN SMALL MAMMALS TO REPEATED EXPOSURE TO CERCARIAE OF SCHISTOSOMA JAPONICUM: Experiments were conducted during 1949 using cercariae of bird and human schistosomes to determine whether or not cross immunity to repeated exposures of the same species could be produced (3). The results were equivocal except in the case of repeated exposures to cercariae of bird schistosomes where an increase in sensitivity was noted (3). This was further substantiated by re-exposure during 1950 eliciting a marked response in rabbits sensitized the previous year. The 1949 experiments also suggested that rabbits exposed to cercariae of S. japonicum followed by those of S. sturniae reacted less severely than when exposed only to S. sturniae (3). Therefore, it was decided to run a series of experiments re-exposing mice and hamsters to relatively small numbers of cercariae of S. japonicum in an attempt to secure data on whether or not immunity could be developed to these parasites.

Cercariae were obtained from infected snails by maceration of the livers in dechlorinated tap water. Only active cercariae from the surface film were used. Fifteen mice were exposed to 10, 20, and 40 cercariae each at three week intervals; 5 hamsters were exposed to 20, 100, and 200 cercariae each. Fifteen new control mice and 5 control hamsters were used at each exposure and autopsied to recover worms three weeks after exposure. The experimental animals were autopsied 3 weeks after the last exposure. Worms were recovered by a perfusion method (15).

Eleven of the original 15 experimental mice survived until autopsied as did 42 of the 45 control mice. All of the hamsters survived until autopsied. The average per cent of worms recovered was 63.1% from the experimental mice and 72.5% from the hamsters as compared with the average per cent of worms recovered from the controls (Table XVII). However, there was considerable variation in percentage of worms recovered from the experimental mice, taken individually, as opposed to a marked uniformity in the controls. This may be partly explained by the fact that the backs of some of the experimental mice had become scarred after the second and third shaving. It is possible that the cercariae were unable to penetrate this scar tissue.

Table XVII. Comparison of Repeated and Single Infections of Schistosoma japonicum in Mice and Hamsters

	No. of Animals Autopsied		No. Cercariae Used in Exposure		No. Worms Recovered at Autopsy		Average % Worms Recovered From Cercariae Used	
	Mice	Hamsters	Mice	Hamsters	Mice	Hamsters	Mice	Hamsters
Experimental (Triple Infection)	11	5	770	1,600	241	410	31.29	25.62
Control (Single Infection)	42	15	930	1,600	461	565	49.57	35.31
Per cent Recovery of Experimental to Control Animals							63.1	72.5

THE EPIDEMIOLOGY OF SCHISTOSOME DERMATITIS (KOGANBYO) IN JAPAN: The study of schistosome dermatitis was begun in 1948, when it was first found along the western end of Lake Shinji in Shimane Prefecture (1). Investigations were continued during 1949 (3) and 1950 which culminated in the present report. In view of the previous rather detailed descriptions in other annual reports (1, 3) the present account will be limited to a summary of the overall results obtained.

As has been previously noted the etiologic agent was discovered by Tanabe (16, 17). During the past summer data were collected on infections in the snail host Polypylis hemisphaerula Benson (Segmentina nitidella) in the Lake Shinji area. The results of the work on the snail hosts during this and previous summers are summarized in Table XVIII. It will be noted that the infections in the snail varied considerably with the locality where collections were made.

Table XVIII. Summary of the Infection of Polypylis hemisphaerula with Cercariae of Gigantobilharzia sturniae Collected During June and July 1949 and June 1950

	Number of <u>Polypylis</u> Collected in:			Number Infected In:			Percent Infected In:		
	June		July	June		July	June		July
Community	1949	1950	1949	1949	1950	1949	1949	1950	1949
Shutto-mura	8,067	9,164	1,968	442	478	32	5.5	5.2	1.6
Hisagi-mura	6	754	297	1	63	4	16.7	8.4	1.3
Hirata-cho		595			57			9.6	
Naoe-mura		1,728	105		59	0		3.4	0.0
Shobara-mura		540	1,206		32	21		5.9	1.7
Nadabun-mura		2,982	1,397		56	19		1.9	1.4
Iwano-mura		391						14.1	
Shinji-machi			600			3			0.5
Total	8,073	16,154	5,573	443	800	79	5.5	5.0	1.4

Through the cooperation of Dr. Tanabe attempts were made to secure data on the distribution and infection of the snail and bird hosts in other areas of Japan as well as on the presence of "koganbyo" (Table XIX). Figure 12 shows the regions where the

snail host, P. hemisphaerula, has been reported and/or collected in Japan. It will be seen from Table XIX that schistosome dermatitis is quite widely distributed throughout the country.

Table XIX. Regions of Japan Where Snails, Birds, or Cases of "Koganbyo" Have Been Found

Area and Prefecture	<u>P. hemisphaerula</u>		Infected Birds			"Koganbyo" Produced Experimentally	Authority
	Present	Infected	Starlings	Sparrows	Wag-Tails		
Lake Shinji Shimane	/	/	/	/	/	*	Tanabe & Mihara Tanabe 1948, 1948a The present paper
Kofu, Yamanashi	/	0	/			-	Tanabe, Miyazaki et al The present paper
Katayama, Hiroshima	/	/	/	/		*	Tanabe and Oda Oda
Notogawa, Lake Biwa, Shiga	/	0	/			-	Tanabe and Oda The present paper
Himeji, Hyogo	/	0	/			-	Tanabe and Oda The present paper
Kojo, Okayama	/	0	/	/, 0		-	The present paper
Lake Kasumi, Ibaraki	0	0	/			-	The present paper
Nagoya, Aichi	/	/	/		/	*	The present paper

Key: / = Present

- = Not produced experimentally

0 = Uninfected

* = Produced experimentally

As the studies are continued it is probable that these records will be further extended, as it is believed that this disease is more widespread than was initially suspected.

PROTECTIVE OINTMENTS AGAINST SCHISTOSOME CERCARIAE: During the previous two years the value of copper oleate had been tested as a protective ointment (1, 3). This substance had been checked in the field as a protective agent against the penetration of the cercariae of the bird schistosome causing "koganbyo" in a joint project with the Japanese National Institute of Health and Kitasato Institute. Subsequently interest was expressed in several other compounds such as dimethyl and dibutyl phthallate, and benzyl benzoate. In 1950 these were re-tested along with two new ointments known as NI and NII.*

* These were furnished through the cooperation and courtesy of Dr. N. Nagano, Kitasato Institute, Tokyo

The NI and NII compounds were tested after the manner of the copper oleate to determine whether or not they held promise as protective ointments. They were tested in vitro against the cercariae of Schistosoma japonicum, as they had proved fairly effective in field trials against bird schistosome cercariae. Six trials were run on each ointment with controls of plain water in which a thin coat of each chemical was placed in the bottom of separate petri dishes and water containing 15 to 25 cercariae added to each dish. At the end of 20 minutes all cercariae in dishes containing ointments were dead, whereas all were alive in the controls.

Twenty mice were used for testing each of the ointments in vivo and 20 as controls. They were exposed to approximately 100 cercariae each. The mice protected with ointment No. 1 (NI) gave only 0.8% recovery of worms, those with ointment No. 2 (NII) gave 1.25% and the controls yielded 68.1% of the number of cercariae used for exposure. This gives a protection rate of 98.83% and 98.1% respectively if the number of worms recovered from the controls is taken to represent 100%.

In the experiment for ointment toxicity 20 mice were used for each ointment. The ointments were applied every other day for five applications. During the course of the experiment three mice died from each group, but the autopsy showed no gross changes due to the ointments. No reaction was evident in the remaining animals.

In the summer of 1950, 123 persons were tested with ointments of copper oleate, benzyl benzoate, dimethyl phthalate, dibutyl phthalate, NI and NII to determine their effectiveness against the cercariae of the bird schistosome. Different groups of people from the endemic "koganbyo" area near Lake Shinji in Shimane Prefecture volunteered as subjects. These persons were working in the muddy rice paddies and thus subjected to considerable abrasive action by the soil. Each leg was painted with the same test ointment. After each individual had worked either four, six, or eight hours in the paddies the protected areas as well as unprotected control areas were checked with viable, mature cercariae. A maximum period of 30 minutes was allowed the control to react and the presence of itching and/or petechiae was interpreted as a positive reaction.

Summary - Table XX shows that copper oleate gave the greatest protection, 95.5%, while NII and dibutyl phthalate were next with 71.4% and 70.6% respectively. However, it is apparent that both NI and dimethyl phthalate give reasonably good protection.

Table XX. Summary of Ointment Field Tests on Paddy Workers in the Lake Shinji Area in 1950

Ointment	Number Tested	Number protected after				Number protected after					
		4 hrs*	6 hrs*	8 hrs	%	4 hrs	%	6 hrs	%	8 hrs	%
Copper oleate	32	6	4	21	94.5	0	0.0	0	0.0	1	4.5
Benzyl benzoate	21	1	4	7	43.8	0	0.0	6	37.5	3	18.8
Dimethyl phthalate	27	3	5	13	68.4	1	5.3	1	5.3	4	21.1
Dibutyl phthalate	22	2	3	12	70.6	0	0.0	4	23.5	1	5.9
N - I	11	3	2	3	50.0	1	16.7	0	0.0	2	33.3
N - II	17	2	1	10	71.4	3	14.3	1	7.1	1	7.1

* These do not indicate failures but rather than the individuals did not return or worked in the paddy only 4 or 6 hours. Failures are reported elsewhere.

EVALUATION OF SKIN TEST ANTIGENS: Introduction - Beginning in 1948 attempts were made to produce antigen from adult specimens of Schistosoma japonicum for use as skin tests in detecting schistosomiasis japonica. This was a cooperative program with the Japanese National Institute of Health, Dr. Sugiura, and later the Yamanashi Medical Research Institute. The best dilution of antigen was found to be 1:10,000. An account of this preliminary work has appeared previously (1).

Methods - Schistosomal antigens were prepared using (a) adult worms and (b) cercariae, using alternate freezing and thawing technique.

Approximately 0.01 ml. was injected in each instance and the test was read 10-15 minutes following the injection. Apparent negatives were checked after approximately 3-4 and 12-16 hours for evidence of delayed reactions. As in the case of previous work done at this and other laboratories a positive test consisted of an increase of 3 mm. in the wheal as compared with the controls.

Antigens from adult Clonorchis sinensis and Paragonimus westermani have also been prepared by essentially the same method. Results of the tests with antigen of C. sinensis gave equivocal results in 1949. No additional testing has been done. (20)

Discussion of Results of Schistosomiasis - In July 1950, a series of 103 school children known to be positive for Schistosoma japonicum and 68 negative controls were skin tested with antigens prepared from the adults and cercariae of S. japonicum. As the cercariae were obtained by crushing the snails, an extract of snail liver was used as an additional control with the cercarial antigen. Previous tests had indicated that the maximum dilution for the antigen prepared from the adult worms was 1:10,000. Consequently this was the dilution arbitrarily selected for both adult and cercarial antigens.

Table XXI is a comparison of the efficacy of the two antigens in detecting cases of schistosomiasis. It will be noted that more false positives were secured among the controls of the cercarial antigen than with the antigen made from the adult worms. Furthermore, the merthiolated saline control gave no false positives in the control group and only one among those afflicted with schistosomiasis japonica. Table XXII summarizes the results obtained with both antigens in both test groups. It will be noted that both the antigen made from the adult worms and that made from the cercariae gave almost identical results as 96, or 93.2%, were positive. However, the saline controls for the adult worm antigen gave better results than did the snail liver extract that served as a control for the cercarial antigen. Consequently, the use of the adult worm antigen is recommended over the cercarial antigen. The results obtained clearly indicate the efficacy of these tests and suggest use of skin tests as a diagnostic tool might well be extended into the areas endemic for schistosomiasis japonica.

The antigen from the adults of S. japonicum is quite specific as only a few of the persons known to have allergies produced false positives. In addition, 34 persons known to harbor Paragonimus westermani were skin tested with S. japonicum antigen made from the adult worms to determine whether or not paragonimiasis cases would produce false positives; none did. Furthermore, as reported previously (3) 51 persons suffering from "koganbyo" were skin tested with S. japonicum antigen and none gave a positive or a delayed reaction in 6, 12, or 24 hours. Fifteen were simultaneously tested with cercariae of G. sturniae on the opposite arm. This is especially interesting as it has been assumed that the antigen of S. japonicum contained some fraction that was common to the entire schistosome group. Furthermore, antigens from trematodes outside of the schistosome groups have yielded positive reactions in the case of S. mansoni infections (18, 19). Consequently, the absence of false positives in this instance was all the more remarkable. The potency of the antigen which was used was, of course, checked on known positive cases of schistosomiasis japonica and nine of the ten persons who were passing eggs in the stool reacted positively. Therefore, the absence of false positives under such conditions suggests that the antigen is more specific than first supposed (3).

Table XXI. Comparison of the Effectiveness of Schistosoma japonicum Antigens

Tests on Schistosomiasis Proven Positive Cases				
Increase in Wheal Size	Adult Antigen	Cercarial Antigen	Liver Extract	Merthiolated Saline
Negative Reactions				
0 - 1 mm.	4	4	77	95
2 mm.	2	2	21	7
Positive Reactions				
3 - 4 mm.	25	19	5	1
5 - 6 mm.	29	23	0	0
7 - 8 mm.	23	27	0	0
9 - 10 mm.	12	18	0	0
11 - 12 mm.	6	6	0	0
13 - 15 mm.	2	4	0	0

Control Tests on Schistosomiasis Proven Negative Cases				
Negative Reactions				
0 - 1 mm.	65	55	52	66
2 mm.	3	6	11	2
False Positive Reactions				
3 - 4 mm.	0	5	5	0
5 - 6 mm.	0	0	0	0
7 - 8 mm.	0	1	0	0
9 - 10 mm.	0	0	0	0
11 - 12 mm.	0	0	0	0
13 - 15 mm.	0	1	0	0

Discussion of Results on Paragonimiasis - During the current year a preliminary pilot test was run on the newly produced paragonimus antigen. A group of 34 known positive cases of paragonimiasis were skin-tested with antigen made from adult specimens of Paragonimus westermani. Antigen dilutions of 1:5,000, 1:10,000 and 1:20,000 were tested. Merthiolated saline served as a control injection while 30 individuals negative for paragonimiasis were utilized as the control group.

From Table XXIII it can be seen that all 34 positive cases were accurately diagnosed with the 1:20,000 antigen dilution. The lesser dilutions, however, were negative for two cases, although in both instances some reaction occurred. On the other hand wheal sizes were in general less for the 1:20,000 dilution. The 1:10,000 dilution gave wheal enlargements equal to those produced by 1:5,000. For the negative control group the two weaker antigens were 100% accurate, as no false positives occurred. The 1:5,000 dilution, however, produced two false positives. Saline controls were all negative in both experimental and control groups.

The stronger antigen of 1:5,000 dilution is the least suitable of the three. An unequivocal choice between the other two dilutions cannot be made on the basis of the data which were collected. The lower percentage of reliability for the 1:10,000 dilution is hardly a significant difference, yet neither are the small wheal sizes produced by the 1:20,000 dilution a distinct limitation.

Summary - These experiments indicate that the antigens made from both S. japonicum and P. westermani are highly specific and hold considerable promise as a diagnostic aid.

Table XXII. Comparison of Skin Test of Adult Worm and Cercarial Antigen of S. japonicum

Increase of Wheal over Control	Adult Antigen Compared with the Saline Control	Cercarial Antigen Compared with the Snail Liver Control
SCHISTOSOMIASIS PROVEN POSITIVE CASES		
NEGATIVE REACTIONS		
0 - 1 mm.	5	6
2 mm.	2	1
POSITIVE REACTIONS		
3 - 4 mm.	25	23
5 - 6 mm.	31	30
7 - 8 mm.	26	24
9 - 10 mm.	8	12
11 - 12 mm.	6	5
13 - 15 mm.	0	2
Total Positive	96	96
Total Cases	103	103
Reliability	93.2%	93.2%
CONTROLS		
NEGATIVE REACTIONS		
0 - 1 mm.	66	61
2 mm.	2	5
Total Negatives	68	66
POSITIVE REACTIONS		
3 - 4 mm.	0	1
5 - 6 mm.	0	0
7 - 8 mm.	0	0
9 - 10 mm.	0	1
11 - 12 mm.	0	0
Total Cases	68	68
Reliability (%)	100%	97.1%

Table XXIII. Results of Skin Tests for Detecting Infections of
Paragonimus westermani

Wheal Increase over control	Antigen Dilutions			Merthiolated Saline	S. japonicum
	1-5,000	1-10,000	1-20,000	Control	

PROVEN INFECTIONS OF P. WESTERMANI

Negative Reactions

0 - 1 mm.		1		34	32
2 mm.	2	1			2

Positive Reactions

3 - 5 mm.	6	7	17
6 - 10 mm.	16	17	12
11 - 15 mm.	6	4	5
16 - 20 mm.	3	3	
20 f	1	1	

Total Positives	32	32	34
Total Cases	34	34	34
Reliability	94.1%	94.1%	100%

CONTROLS - INDIVIDUALS NEGATIVE FOR P. WESTERMANI

Negative Reactions

0 - 1 mm.	28	30	29	30
2 mm.	0		1	

Total Negatives	28	30	30	30
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Positive Reactions

3 - 5 mm.	2	0	0
6 - 10 mm.	0	0	0
11 - 15 mm.	0	0	0
16 - 20 mm.	0	0	0
20 f	0	0	0

Total Cases	30	30	30
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Reliability	93.3%	100%	100%
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THE EFFECTIVENESS OF CERTAIN ANTHELMINTICS IN THE JAPANESE: Introduction -

Much has been written concerning the efficacy of various vermifuges but there are few comparative data involving tests on several different drugs run on the same or nearly identical groups of people. Over the years santonin has been the drug of choice in Japan for Ascaris. The second choice frequently appears to have been an extract of an alga, Digenes simplex. Recently the manufacture of hexylresorcinol was undertaken in Japan, but medical authorities were slow to utilize it, due in part to reported unpleasant side effects. Since its production became possible at a reasonable cost and in sufficient quantity and quality to meet the needs of the Japanese it was hoped that the government and the physicians would accept this product. This was particularly desirable as the drug is considered to be highly effective against Ascaris lumbricoides and moderately so against hookworm and to a lesser extent whipworm (Trichuris trichiura). This was important since these are the three most common helminths to be found in the Japanese.

In order to secure comparative data on the relative effectiveness of several vermifuges potentially available to the Japanese, a cooperative study was undertaken by the Japanese National Institute of Health, the 406th Medical General Laboratory and officials of Yamanashi Prefecture. The drugs which were selected included the alga, Digenes simplex, santonin, hexylresorcinol of Japanese and American manufacture, and a new product, hetrazan. The Kofu valley in Yamanashi-ken was selected because of the variety, high incidence, and density of the parasitic infections, and their relatively uniform distribution throughout the communities.

Methods - In order that statistically significant results be secured, biometricians were consulted concerning the selection and size of the sample. In this connection it was determined that 200 persons parasitized with Ascaris should be treated with each test drug followed in two hours by a sodium sulfate purge, and, in addition, a group of 200 controls would be given a purge, thus simulating all of the conditions except the administration of the drug (Table XXIV).

The AMS III technique (27) was used throughout since it is highly effective in study of all the helminth eggs. Two pre-treatment examinations provided a base line for comparison with two examinations made three weeks after treatment. From these data the per cent effectiveness of the drug, i.e. complete cure, could be ascertained, and also the per cent reduction in the parasite burden as evaluated by a parasite density index (1, 3). In addition stools were examined at the two and four week intervals after treatment.

A summary of the regimen of treatments is given in Table XXIV. Whenever tablets or capsules were administered, the mouth was always checked to be sure they had been swallowed. In all instances approximately 20 grams of crystalline sodium sulfate was given as a purge two hours after the last dose of the drug.

A mimeographed check sheet was prepared for recording side effects from the drugs. Entries for light, moderate and severe reactions were made at intervals of 4, 12, 24, and 48 hours after treatment. A control entry was made for any symptoms which occurred before treatment.

Effectiveness of the Drugs - The results indicated clearly the superiority of both Japanese and American hexylresorcinol over any of the other drugs that were tested for the removal of Ascaris lumbricoides (Table XXV). In the case of the former 73.5% were entirely freed of worms while the American product eliminated 85% of these parasites. When the change in the parasite density index was considered it was noted that the overall reduction was 88.5% and 96% respectively. These results were superior to those obtained with santonin and Digenes simplex which only gave 24.5% and 19% clearance of worms and a reduction of the worm burden of 50% and 44.4% respectively. Hetrazan was not effective against Ascaris in the dosages used.

As regards hookworm, the figures show that the Japanese product removed all worms in 42.3% of those infected, whereas the figure was 29.7% for the American drug. However, the decrease in the parasite density index was about the same for both. Reductions with hetrazan were less, and neither of the other two drugs was effective.

Table XXIV. Summary of Treatment Schedule

JAPANESE HEXYLRESORCINOL (Based on Ministry of PH&M's recommendations (21))

1. Light supper or fasting night before treatment.
2. No breakfast, small amounts of water may be taken.
3. Give 1 gm. (5 tablets) with water to adults. (4 tablets 8-12 years; 3 tablets 6-7 years). These must not be chewed.
4. Two hours after dose give sodium sulfate purge.
5. Food is proscribed for five hours after taking drug.

AMERICAN HEXYLRESORCINOL (Based on Faust (22); Mackie, Hunter, and Worth (23))

1. Light supper or fasting night before treatment.
2. No breakfast, small amounts of water may be taken.
3. Give 1 gm. (5 tablets) with water to adults.
4. Two hours afterwards give sodium sulfate purge.
5. Food is proscribed for five hours after taking drug.

HETRAZAN (Lederle's recommendations (24))

1. Eat normally.
2. Give adults 2 tablets (a total of 6-10 mgms per kgm) 3 times per day for five days.
3. Two hours after last dose give saline (sodium sulfate) purge.
4. Total dosage is about 1,400 mg.

DIGENEA SIMPLEX (Based on Morishita (25))

1. Normal supper night before.
2. Give 50 ml.* after breakfast and lunch (two times per day* for 3 days. Can be given once a day as 100 ml. if tolerated. Total dose is 30 gms of raw product.

* Take 10 gms. of Digenea, raw, in 200 ml. water. Boil to 100 ml. and use 50 ml. per dose. This is prepared fresh each day. It is believed that raw Digenea is better than prepared pills (based on extracts).

SANTONIN (Based on Ministry of PH&M's recommendations (21))

1. Light supper early in evening.
2. Give 0.10 gm. before going to bed with an ordinary amount of water.
3. Early next morning give 0.06 gm. If there are any complications, do not give this dose. Total dose 0.16 gm.
4. Purgation or meal at two hours after (3). Purge is 20 gms. (10-20) sodium sulfate.

The absence of any decrease in incidence for whipworm (*T. trichiura*) was unusual, as hexylresorcinol is reported to be moderately efficacious in the removal of these parasites. However, there was a noticeable drop in the parasite density index by both hexylresorcinols.

Since a Stoll Dilution Egg Count Equivalent was calculated for our "plus" egg count categories after the method of Hood (26) it has been possible to interpolate a probable worm burden for the cases of ascariasis, trichuriasis and hookworm infections. For example, the 200 individuals who received Japanese hexylresorcinol were calculated to harbor 4095 ascarids, whereas after treatment the number was determined to be 805, a reduction of 80.3%, which compares favorably with the calculation of 88.5% reduction in the parasite density index.

Table XXV. Results of Treatment with Several Anthelmintics

Drug Used	No. of Infected Cases	Percent Completely Freed of Worms	Parasite Density Index Pre-treatment	Post-treatment	Percent Reduction of Density Index
A S C A R I S					
Hexylresorcinol (Jap.)	200	73.5	122	14	88.5
Hexylresorcinol (Amer.)	205	84.9	125	5	96.0
Hetrazan	205	9.8	114	61	46.5
<u>Digenea simplex</u>	204	19.1	117	65	44.4
Santonin	205	24.5	98	49	50.0
Controls	204	5.9	117	80	31.6
W H I P W O R M					
Hexylresorcinol (Jap.)	182	4.9	128	97	24.2
Hexylresorcinol (Amer.)	191	3.7	91	80	12.1
Hetrazan	200	0.0	192	146	24.0
<u>Digenea simplex</u>	194	1.5	123	138	increased
Santonin	185	1.1	94	97	increased
Controls	200	1.5	145	128	11.7
H O O K W O R M					
Hexylresorcinol (Jap.)	104	42.3	26	7	73.1
Hexylresorcinol (Amer.)	145	29.7	29	8	72.4
Hetrazan	118	24.6	23	12	47.9
<u>Digenea simplex</u>	112	22.3	22	18	18.2
Santonin	120	21.7	28	25	10.7

Tabulations were made to compare the effectiveness of the drugs in light, moderate and heavy infections. In the case of ascaris the light infections were more apt to be completely eliminated than were the heavy ones. This was true for all the drugs. Although there were very few heavy hookworm infections for comparison, a considerable difference was noted between light and moderate infections. Part of this difference must be attributed to the increased chance of missing infections as the number of worms and eggs becomes reduced. American hexylresorcinol was noticeably more effective than the Japanese product in achieving complete removal of worms in heavy infections. Although the difference between the overall efficacy of these two drugs is not great, the American hexylresorcinol is more effective. However, the level of efficiency for the Japanese drug is considerably above that of santonin and Digenea.

Side Effects of Treatment - Santonin was the only drug which produced relatively severe side reactions. Of 205 so treated, 147 complained of "yellow vision". As noted by various authors, this manifestation occurs with only slightly more than the therapeutic dose (23); there is only a minimal "margin of safety" between the toxic and therapeutic doses. Other symptoms reported along with "yellow vision" suggest that the maximum dosage was approached or exceeded in this investigation, so that increased efficacy could not be hoped for by merely increasing the dosage.

The reactions resulting from the two hexylresorcinols did not differ greatly, except that more persons reported nausea or vomiting after the Japanese drug. In the case of the latter, 41 experienced nausea and 9 vomiting after 4 hours; 5 cases with each symptom were severe. The corresponding numbers for the American hexylresorcinol were 23 and 4,

none of which were severe; similar numbers of those receiving the purge only also reported nausea and vomiting. About half the individuals taking each make of hexylresorcinol reported slight or moderate headache, in contrast to one-fifth of the controls. Abdominal pain to a slight or moderate degree was reported by about one-fourth, of which one-third can be discredited by the controls. For some reason the purge was apparently less effective for the group taking Japanese hexylresorcinol, as indicated by the number who reported diarrhea. This may explain why it was less effective than the American product.

The groups taking hetrazan and Digenea simplex did not experience marked ill-effects when compared with the controls.

None of the 1019 individuals receiving one of the anthelmintics experienced side reactions to a critical degree. Further, it is evident that the purge accounted for some of the symptoms; some persons reported them even before taking the drugs. Although Japanese hexylresorcinol made a few people ill, the overall picture clearly supports the contention that it is a safer and a considerably more effective drug against ascaris and hookworm than santonin and Digenea.

Summary - Five drugs, Japanese hexylresorcinol, American hexylresorcinol, hetrazan, Digenea simplex, and santonin were tested in similar lots of approximately 200 persons each from communities in the Kofu valley, Yamanashi-ken. Hexylresorcinol is outstandingly more effective in the removal of Ascaris lumbricoides than either santonin or Digenea simplex. Hexylresorcinol is quite effective in reducing hookworm burden. The per cent reduction of the parasite density index is about 73%, which compares with less than 11% for santonin and 18% for Digenea simplex. Hexylresorcinol, while not strikingly effective against whipworm (Trichuris trichiura) is nevertheless more efficacious than any of the other drugs tested. By interpolating our data into the Stoll Egg Dilution Count it was possible to secure figures on the probable actual number of Ascaris lumbricoides that were eliminated. Side effects were not so marked in the case of the hexylresorcinols as in santonin.

EPIDEMIOLOGIC STUDIES IN THE FAR EAST:

OBSERVATIONS ON RE-INFECTIONS BY ASCARIS LUMBRICOIDES: It has been shown by students of public health that the treatment of the infected population in endemic areas will seldom, if ever, result in the elimination of helminth parasites (22, 23, 26, 36, 37, 38). This is well known to be the case in Japan, yet there is tremendous pressure upon the governmental agencies to provide treatment annually for certain population groups such as school children. In an attempt to determine how quickly Ascaris lumbricoides returns after treatment 113 persons who had been freed of Ascaris were examined monthly after treatment. Each month fresh stool specimens were collected and sent to this laboratory. These specimens were examined by the AMS III technique by the same personnel who made the original examinations. Only persons known to be free of A. lumbricoides on the basis of multiple stool examinations were used. The general population of these two communities that were used in these studies had 82.0% and 74.4% of ascariasis respectively.

It will be seen that at the end of the second month only 6.2% were infected (Table XXVI). This was tripled the following month and that figure doubled by the fourth month. By this time the parasite density index had increased over threefold (Table XXVI).

It will also be noted that there is not a steady increase in the incidence and density of ascariasis. This may be explained in part by the fact that during the eighth and ninth months the people ate many sweet potatoes. At such times it is difficult to obtain completely satisfactory stool specimens, due to the consistency of the stool. Furthermore during this same period 9 persons of this group treated themselves with santonin or some other anthelmintic. An additional point lies in the fact that two persons became negative for Ascaris in the eighth month, five in the ninth month, two in the tenth and one in the eleventh month. If these were moderately infected or heavily infected persons it might result in a lowering of the parasite density index even though the actual incidence of ascariasis increased slightly (67.3% - 70.0%). This could be explained on the grounds that the newly acquired infections were probably considerably lighter than those that were treated. So little is known of the length of life of Ascaris lumbricoides in the human host that it cannot be stated whether the decrease in incidence during months eight and nine was real or apparent. Table XXVI shows by comparing the parasite density

Table XXVI. Summary of Re-infections by Ascaris lumbricoides During a 10 Month Period in 113 Individuals

Month after Treat- ment	Month	Egg Count Categories						Total	Parasite Density %	Index
		1	2	3	4	5				
		1-9	10-49	50-99	100-199	200-399	400			
2	March	2	4	0	0	1	0	7	6.2	61
3	April	5	12	4	1	2	0	24	21.2	60
4	May	11	6	4	7	8	11	47	41.6	202
5	June	15	8	2	8	5	4	42	37.2	123
6	July	6	7	13	15	15	14	70	61.9	214
7	August	14	17	13	16	7	9	76	67.3	139
8	September	11	31	8	7	1	0	58	51.3	51
9	October	17	25	8	5	6	6	67	59.3	104
10	November	17	28	9	8	8	6	76	67.3	108
11	December	10	32	16	10	7	4	99	70.0	99

Summary - The re-infection of Ascaris-free individuals gradually built up during a period of eleven months to 70% while the greatest worm burden occurred 6 months after treatment.

AN EPIDEMIOLOGIC SURVEY OF SHIZUOKA PREFECTURE, JAPAN: Introduction - A parasite survey was made in Shizuoka Prefecture during August and September, 1950. The objectives of the project included: (i) collection of incidence data and parasite densities for all intestinal parasites; (ii) delineation of schistosomiasis foci in the Numazu endemic center; (iii) an evaluation of the skin testing technique as a means of recognizing infections of the lung fluke, Paragonimus westerni; and (iv) collection of blood specimens to ascertain the status of filariasis, previously reported for the Shizuoka area.

Methods - Twenty-two communities were selected which it seemed should furnish data pertinent to the above objectives. This necessitated, of course, recognizing previously identified centers of schistosomiasis, paragonimiasis and filariasis. For other parasites the selection of communities was without a priori knowledge of incidence and parasite density. Areas beyond the limits of the above endemic centers were also included.

Techniques and procedures followed included the AMS III (27) and 406th MGL (39) ether concentration techniques for eggs and cysts, the Graham scotch tape method (23) for recovering pinworm eggs, a modified Knott technique (23) for collection of blood specimens for the recovery of microfilariae, and the skin testing procedure for paragonimiasis which was previously used by this department for schistosomiasis. A systematic search, consistent with snail habits and distribution of human cases of schistosomiasis, was made for snails over approximately eight square miles.

Findings by Stool - A total of 2273 individuals were examined, of which 94.7% were parasitized; 92.8% harbored helminths and 33.6% were found to be infected with protozoa (Table XXVII). Ascaris and whipworm occurred in 79.9% and 56.6% respectively; the incidence of pinworm was 55.5% for 440 children. The overall prevalence of hookworm was 27.5%. The occurrence of Trichostrongylus sp. was relatively low, quite in contrast to the adjacent prefecture of Yamanashi. The overall incidence of protozoa was quite low for the survey as a whole. Endamoeba histolytica was found in only 4.4% of those examined while for E. coli and Endolimax nana the percentages were 22.5 and 13.5 respectively.

The most prevalent parasites and those of special interest are given by community in Table XXVIII. The incidence range for ascaris, whipworm, and hookworm in the various communities was considerable, 60.5% to 93.0% for ascaris, 34.7% to 85.0% for whipworm, and 4.0% to 56.7% in the case of hookworm. Pinworm was prevalent in most communities, with as many as 16 of 20 children at Takaoka and 18 of 25 at Ema being found infected by one scotch tape test. Cases of schistosomiasis were found in six communities. The highest incidence of 26.0% existed at Kanaoka which is near the eastern limits of the

endemic center. Findings of 3.0% to 5.0% occurred in three villages near the western end, for which Yoshiwara can be used arbitrarily as the geographical land mark. Selected population centers in the paragonimiasis endemic center included Tsukamoto, Hita, and Ema, where 12.5%, 16.4%, and 4.0% respectively were infected. A few cases occurred beyond the recognized endemic limits, particularly at Sudo where five of 101 persons were found infected by stool examination. Metagonimus yokogawai was found in 8 of 104 individuals, or 7.7%, at Motoyoshiwara. It occurred in 2.0% to 4.0% of four other communities. The overall infection rate exceeded that of Clonorchis sinensis which usually predominates over Metagonimus.

Table XXVII. Summary of Intestinal Parasites for 22 Communities in Shizuoka Prefecture

	<u>No. Infected</u>	<u>Incidence</u>
No. of persons examined	2273	
No. parasitized	2153	94.7
No. with helminths	2110	92.8
No. with protozoa	763	33.6
Helminths:		
<u>Ascaris lumbricoides</u>	1815	79.9
<u>Trichuris trichiura</u>	1332	58.6
Hookworm	625	27.5
<u>Trichostrongylus sp.</u>	199	8.8
* <u>Enterobius vermicularis</u>	244/440	55.5
<u>Schistosoma japonicum</u>	40	-
<u>Paragonimus westermani</u>	43	-
<u>Metagonimus yokogawai</u>	27	1.2
<u>Clonorchis sinensis</u>	25	1.1
Other heterophyids	36	1.6
Echinostome	2	0.1
<u>Fasciola hepatica</u>	1	0.04
<u>Hymenolepis nana</u>	2	0.1
<u>Hymenolepis diminuta</u>	2	0.1
Protozoa:		
<u>Endamoeba histolytica</u>	100	4.4
<u>Endamoeba coli</u>	512	22.5
<u>Endolimax nana</u>	307	13.5
<u>Iodamoeba butschlii</u>	13	0.6
<u>Giardia lamblia</u>	90	4.0
<u>Chilomastix mesnili</u>	4	0.2

* Scotch tape swab

The incidence of E. histolytica was only 1.0% in three of the 22 communities and 5.0% or less in 14 communities. In no instance was the rate as high as 10.0%. Other protozoan infections were also moderate and their fluctuations in the various communities coincided in general with E. histolytica.

Status of Schistosomiasis in the Numazu Area - Wright et al (5) described the endemic center of schistosomiasis in the Numazu area of Shizuoka Prefecture as "comprising about 10 square miles and extending from 1 mile east of the town of Numazu westward as far as Yoshiwara". The above authors examined children in one village (Sudo). Their survey for snails was also limited to this village, where they found them in one of two areas searched (Figs. 13, 14).

In the current investigation, stool surveys were made in 14 communities bounding the above described area. Also 470 individuals were skin tested with adult worm antigen of Schistosoma japonicum, and a systematic search for snails was made over a large portion of the area. The findings indicate that two prominent foci of snails and human infections prevail, one near each end of the endemic center recognized by Wright et al (5). To the east, the focus is immediately north of Numazu City in the community of Kanaoka, while the one to the west is centered in the village of Sudo, extending into Ukashima and Motoyoshiwara.

Table XXVIII. Intestinal Parasitism in the Various Communities
Surveyed in Shizuoka Prefecture

	Tsukamoto- buraku		Hita- buraku		Ema- mura		Ganyudo- ku		Banba ku	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. Examined	104		104		124		100		105	
No. with parasites	100	96.2	96	92.3	116	93.6	90	90.0	92	87.6
No. with helminths	97	93.3	94	90.4	115	92.7	86	86.0	91	86.6
No. with protozoa	37	35.6	34	32.7	25	20.2	39	39.0	27	25.7
Helminths:										
<u>Ascaris</u>	75	72.1	78	75.0	101	81.5	69	69.0	77	73.3
Whipworm	59	56.7	54	51.9	43	34.7	49	49.0	49	46.6
Hookworm	59	56.7	52	50.0	18	14.5	11	11.0	13	12.4
<u>Trichostrongylus</u> sp.	12	11.5	17	16.4	3	2.4	5	5.0	11	10.5
<u>S. japonicum</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>H. nana</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>H. diminuta</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>Clonorchis</u>	1	1.0	1	1.0	1	0.8	0	0.0	1	1.0
<u>Metagonimus</u>	0	0.0	0	0.0	0	0.0	2	2.0	1	1.0
<u>Paragonimus</u>	13	12.5	17	16.4	5	4.0	0	0.0	0	0.0
<u>Heterophyes</u> sp.	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
Pinworm in stool	6	5.8	7	6.7	8	6.5	10	10.0	10	9.5
Pinworm swab	9/16	56.3	6/14	42.9	18/25	72.0	16/25	64.0	12/25	48.0
<u>Rhabditis</u> sp.	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
Echinostome	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Unidentified fluke	0	0.0	0	0.0	1	0.8	0	0.0	0	0.0
<u>F. hepatica</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Protozoa:										
<u>E. histolytica</u>	2	1.9	1	1.0	1	0.8	5	5.0	2	1.9
<u>E. coli</u>	25	24.0	21	20.2	16	12.9	25	25.0	21	20.0
<u>E. nana</u>	14	13.5	13	12.5	4	3.2	6	6.0	3	7.6
<u>Giardia</u>	9	8.7	3	2.9	8	6.5	5	5.0	5	4.8
<u>Chilomastix</u>	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>Iodamoeba</u>	0	0.0	0	0.0	0	0.0	1	1.0	1	1.0

Table XXVIII Continued

	Izumi- mura		Nagaizumi- mura		Ashitaka- mura		Ooka		Kanaoka ku	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. Examined	100		118		101		100		100	
No. with parasites	97	97.0	111	94.1	93	92.1	97	97.0	97	97.0
No. with helminths	97	97.0	109	92.4	92	91.1	95	95.0	97	97.0
No. with protozoa	41	41.0	40	33.9	35	34.7	33	33.0	32	32.0
Helminths:										
<u>Ascaris</u>	90	90.0	93	78.8	83	82.2	82	82.0	93	93.0
Whipworm	77	77.0	69	58.5	51	50.5	66	66.0	69	69.0
Hookworm	18	18.0	38	32.2	35	34.7	35	35.0	30	30.0
<u>Trichostrongylus</u> sp.	3	3.0	18	15.3	0	0.0	19	19.0	2	2.0
<u>S. japonicum</u>	0	0.0	0	0.0	0	0.0	0	0.0	26	26.0
<u>H. nana</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>H. diminuta</u>	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
<u>Clonorchis</u>	1	1.0	0	0.0	1	1.0	2	2.0	0	0.0
<u>Metagonimus</u>	1	1.0	1	0.9	0	0.0	0	0.0	0	0.0
<u>Paragonimus</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>Heterophyes</u> sp.	0	0.0	3	2.5	2	1.9	0	0.0	1	1.0
Pinworm in stool	6	6.0	6	5.1	11	10.9	6	6.0	2	2.0
Pinworm swab	13/25	52.0	7/24	29.2	12/19	63.1	17/25	68.0	12/22	54.5
<u>Rhabditis</u> sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Echinostome	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
Unidentified fluke	0	0.0	0	0.0	1	1.0	0	0.0	1	1.0
<u>F. hepatica</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Protozoa:										
<u>E. histolytica</u>	9	9.0	5	4.2	6	5.9	3	3.0	6	6.0
<u>E. coli</u>	32	32.0	33	27.9	26	25.8	24	24.0	22	22.0
<u>E. nana</u>	22	22.0	15	12.7	12	11.9	7	7.0	13	13.0
<u>Giardia</u>	2	2.0	7	5.9	3	2.9	7	7.0	2	2.0
<u>Chilomastix</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>Iodamoeba</u>	3	3.0	1	0.9	0	0.0	0	0.0	0	0.0

Table XXVIII. Continued

	Katahama		Ukishima- mura		Sudo-mura		Yoshinaga- mura		Yoshiwara- shi	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. Examined	100		100		101		107		106	
No. with parasites	98	98.0	99	99.0	100	99.0	103	96.3	94	88.7
No. with helminths	93	93.0	99	99.0	100	99.0	101	94.4	92	86.8
No. with protozoa	46	46.0	38	38.0	21	20.8	37	34.6	37	34.9
Helminths:										
<u>Ascaris</u>	84	84.0	93	93.0	87	87.0	91	85.0	71	67.0
Whipworm	43	43.0	61	61.0	57	57.0	69	64.5	59	55.7
Hookworm	18	18.0	26	26.0	39	39.0	29	27.1	32	30.2
<u>Trichostrongylus</u> sp.	6	6.0	1	1.0	8	8.0	3	2.8	9	8.5
<u>S. japonicum</u>	1	1.0	3	3.0	5	5.0	0	0.0	0	0.0
<u>H. nana</u>	0	0.0	1	1.0	1	1.0	0	0.0	0	0.0
<u>H. diminuta</u>	0	0.0	0	0.0	0	0.0	1	0.9	0	0.0
<u>Clonorchis</u>	3	3.0	5	5.0	1	1.0	0	0.0	0	0.0
<u>Metagonimus</u>	0	0.0	3	3.0	3	3.0	4	3.7	0	0.0
<u>Paragonimus</u>	0	0.0	0	0.0	5	5.0	2	1.9	0	0.0
<u>Heterophyes</u> sp.	0	0.0	4	4.0	14	14.0	3	2.8	0	0.0
Pinworm in stool	7	7.0	6	6.0	3	3.0	5	4.7	2	1.9
Pinworm swab	12/22	54.5	11/20	55.0	6/22	27.3	7/15	46.7	16/23	69.6
<u>Ahabcitis</u> sp.	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Echinostome	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
Unidentified fluke	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>F. hepatica</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Protozoa:										
<u>E. histolytica</u>	9	9.0	6	6.0	5	5.0	5	4.7	7	6.6
<u>E. coli</u>	30	30.0	22	22.0	11	10.9	18	16.8	21	19.8
<u>E. nana</u>	16	16.0	20	20.0	9	8.9	12	11.2	18	17.0
<u>Giardia</u>	2	2.0	3	3.0	2	2.0	3	2.8	7	6.6
<u>Chilomastix</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>Iodamoeba</u>	0	0.0	0	0.0	1	1.0	0	0.0	1	0.9

Table XXVIII. Continued

	Harada- mura		Fuji- machi		Takaoka- machi		Fujimiya- shi		Motoyoshi- wara-mura	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. Examined	100		107		100		107		104	
No. with parasites	95	95.0	104	97.2	97	97.0	104	97.2	97	93.3
No. with helminths	90	90.0	103	96.3	97	97.0	104	97.2	96	92.3
No. with protozoa	47	47.0	33	30.8	27	27.0	43	40.2	29	27.9
Helminths:										
<u>Ascaris</u>	82	82.0	88	82.2	80	80.0	86	80.4	88	84.6
Whipworm	58	58.0	77	65.4	71	71.0	91	85.0	63	60.6
Hookworm	13	13.0	50	46.7	23	23.0	46	43.0	18	17.3
<u>Trichostrongylus</u> sp.	4	4.0	14	13.1	5	5.0	23	21.5	4	3.8
<u>S. japonicum</u>	0	0.0	0	0.0	0	0.0	0	0.0	4	3.8
<u>H. nana</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>H. diminuta</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>Clonorchis</u>	1	1.0	1	0.9	0	0.0	3	2.8	1	0.9
<u>Metagonimus</u>	0	0.0	1	0.9	0	0.0	0	0.0	8	7.7
<u>Paragonimus</u>	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>Heterophyes</u> sp.	0	0.0	2	1.8	1	1.0	0	0.0	5	4.8
Pinworm in stool	4	4.0	1	0.9	2	2.0	2	1.8	2	1.9
Pinworm swab	14/22	63.6	12/18	66.7	16/20	80.0	5/15	33.3	8/18	44.4
<u>Rhabditis</u> sp.	0	0.0	0	0.0	0	0.0	0	0.0	1	0.9
Echinostome	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Unidentified fluke	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0
<u>F. hepatica</u>	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
Protozoa:										
<u>E. histolytica</u>	7	7.0	3	2.8	2	2.0	7	6.5	5	4.8
<u>E. coli</u>	31	31.0	23	21.5	21	21.0	26	24.3	21	20.2
<u>E. nana</u>	29	29.0	17	15.9	9	9.0	18	16.8	14	13.5
<u>Giardia</u>	5	5.0	2	1.8	5	5.0	4	3.7	0	0.0
<u>Chilomastix</u>	0	0.0	1	0.9	0	0.0	0	0.0	0	0.0
<u>Iodamoeba</u>	1	1.0	1	0.9	0	0.0	2	1.8	0	0.0

Table XXVIII. Continued

	Haramachi		Ote-machi	
	No.	%	No.	%
No. Examined	99		86	
No. with parasites	94	94.9	79	91.9
No. with helminths	87	87.9	75	87.2
No. with protozoa	36	36.4	26	30.2
Helminths:				
<u>Ascaris</u>	72	72.7	52	60.5
Whipworm	59	59.6	45	52.3
Hookworm	4	4.0	18	20.9
<u>Trichostrongylus</u> sp.	11	11.1	21	24.4
<u>S. japonicum</u>	1	1.0	0	0.0
<u>H. nana</u>	0	0.0	0	0.0
<u>H. diminuta</u>	0	0.0	0	0.0
<u>Clonorchis</u>	2	2.0	0	0.0
<u>Metagonimus</u>	3	3.0	0	0.0
<u>Paragonimus</u>	1	1.0	0	0.0
<u>Heterophyes</u> sp.	0	0.0	0	0.0
Pinworm in stool	11	11.1	2	2.3
Pinworm swab	12/18	66.7	3/7	42.9
<u>Rhabditis</u> sp.	0	0.0	0	0.0
Echinostome	0	0.0	0	0.0
Unidentified fluke	0	0.0	0	0.0
<u>F. hepatica</u>	0	0.0	0	0.0
Protozoa:				
<u>E. histolytica</u>	1	1.0	3	3.5
<u>E. coli</u>	30	30.3	13	15.1
<u>E. nana</u>	18	18.2	13	15.1
<u>Giardia</u>	2	2.0	4	4.7
<u>Chilomastix</u>	2	2.0	0	0.0
<u>Iodameba</u>	1	1.0	0	0.0

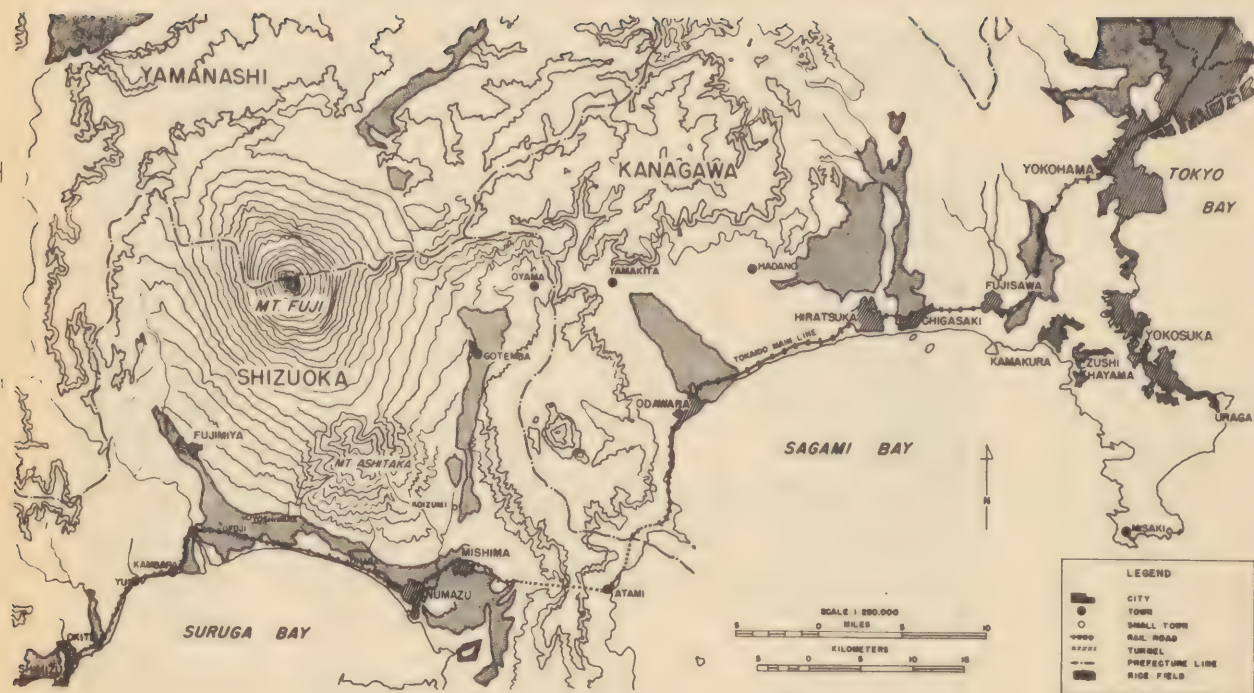


FIGURE 13 — MAP OF SHIZUOKA PREFECTURE SHOWING
AREA ENDEMIC FOR SCHISTOSOMIASIS JAPONICA.

Stool examinations in and to the south and east of Numazu City did not reveal cases of schistosomiasis. In Kanaoka, immediately to the north of Numazu, the incidence was 26.0%. Progressing westward no cases were found at Ashitaka on the back road, while on the bay-side road the incidence was only 1.0% at both Katahama and Hara. Then at Ukishima and Sudo the incidence increased to 3.0% and 5.0% respectively, and was 3.8% at Motoyoshiwara, the counterpart on the bay-side road. Beyond Sudo, including Yoshinaga, Harada and Yoshiwara, cases positive by stool were not found.

A careful search for snails was made over the entire endemic area. Snail distribution was limited to the two foci of human infections mentioned above. At Kanaoka they were found at the edge of a number of rice paddies. Within this focus their distribution was spotty, but they were present in considerable numbers at one point. The colony did not appear to extend into the adjacent northern perimeter of Numazu City. In the western focus snails were found at three well separated points, of which two were in Sudo and one in the village of Ukishima. At the latter place only two snails were found, while in Sudo both colonies were relatively large, one solidly covering 60 to 75 square meters. Only at Kanaoka were snails found which had successfully maintained themselves in areas which were entirely under cultivation. However, they were not typically found in ditches, which serve chiefly for drainage rather than irrigation in the endemic center. At Sudo the habitat was essentially utilized marsh areas, although some snails were found at the edge of adjoining rice paddies. A total of 594 snails were collected and dissected, of which none were infected with Schistosoma japonicum.

Skin tests indicate that schistosomiasis is considerably less prevalent in children than in adults. Young adults (16 to 30 years) also had lower infection rates than older adults. In Shizuoka, males were much more frequently infected than females. These findings were quite in contrast to those for the endemic center in Yamanashi Prefecture.

Status of Filariasis in Shizuoka Prefecture - A total of 350 blood specimens from 6 communities were taken for recovery of microfilariae according to the Knott technique; none of the specimens were positive. Japanese literature includes comments regarding the existence of filariasis in the general area of Yoshiwara where our specimens were collected. If filariasis does exist in the areas surveyed, its endemicity at present is at a very low level, and not of public health significance.

Summary - An epidemiologic survey was made of 2273 Japanese from 22 communities of Shizuoka Prefecture. A total of 94.7% of the people were parasitized. The incidence of helminthiasis varied markedly within the area surveyed. The overall incidence of protozoa was low. Skin tests for schistosomiasis revealed a higher incidence in the older people. There was no evidence of filariasis found in 350 people on whom blood smears were made. On the island of Hachijo Wuchereria malayi occurs (50).

PARASITOLOGIC FINDINGS ON AMERICAN TROOPS FROM KOREA: An investigation was begun to determine the extent to which American troops have contracted parasitic diseases as a result of combat experiences in Korea. The project was to include an examination of: (i) American prisoners of war; (ii) personnel from combat units; and (iii) personnel from rear echelons. The following report is based upon a group of approximately 100 Americans, some of whom were prisoners of war. The prisoners of war comprised two groups, the first including individuals who were taken prisoner chiefly along the Pusan perimeter during July and August 1950, and who either escaped, were released, or were left as dead. Most had been taken as far north as Pyongyang, and returned to American units about mid-October. The second group were captured later in North Korea by the Chinese and released after a short period of about 3 weeks as captives.

The parasitic findings are tabulated in Table XXIX. Eleven of 66 prisoners of war were parasitized. None were infected with ascaris, 4 had a single helminth infection involving hookworm, and 1 man had a triple infection of hookworm, whipworm, and Trichostrongylus sp. Three of 5 hookworm cases were from areas in the United States where hookworm is known to occur. The individual with the triple infection was from New York State (urban) and had not spent time in Japan or other parts of the orient prior to service in Korea. It seems most probable that his infections were contracted in Korea.

Four of the 66 prisoners of war were found to be infected with Endamoeba histolytica, a figure which is regarded as being within the normal limits for amebiasis. Of 29 men evacuated from Korea because of wounds, 8 had parasites, there were 2 infections of ascaris, 1 of whipworm, and 3 men were found to be infected with E. histolytica. Of 56 individuals examined for pinworm by the scotch tape technique, none were found to be infected. In the 2 groups 103 persons were examined for filariasis using the modified Knott technique (23). These were all negative.

Table XXIX. Parasitological Findings on Americans from Korea During 1950

	For Intestinal Parasites		
	Prisoners of War	Combat Personnel	Total
No. Examined	66	29	95
No. Parasitized	11	8	19
Helminths:			
<u>Ascaris lumbricoides</u>	0	2	2
<u>Trichuris trichiura</u>	1	1	2
Hookworm	5	0	5
<u>Trichostrongylus</u> sp.	1	0	1
Protozoa:			
<u>Endamoeba histolytica</u>	4	3	7
<u>Endamoeba coli</u>	3	3	6
<u>Endolimax nana</u>	1	2	3
<u>Iodamoeba butschlii</u>	0	2	2
<u>Giardia lamblia</u>	1	0	1
	<u>No. Examined</u>	<u>No. Positive</u>	
Examined for pinworm by scotch tape	56	0	

It is surprising that the figure for E. histolytica is so low when one considers the probable exposure of our troops to sources of contamination. The fact that many of these people were on suppressive chloroquine and that chloroquine is known to be effective in the treatment of amebic abscess (40, 41) raises the question of a possible correlation. To be sure, according to the current literature (42) it is not believed that the plasma concentration of chloroquine at the suppressive dose is sufficiently high to have a deleterious effect on E. histolytica. However, this possibility should not be entirely overlooked.

Summary - The number of American evacuees from Korea which has been examined for intestinal parasites is still too small to allow any valid conclusions to be drawn. However, there is no evidence to suggest excessive parasitism in combat personnel or prisoners of war from Korea.

MALARIA IN KOREA: Introduction - The fact that malaria is endemic in Korea has been known since the time of the Japanese occupation, and possibly before (43). There is very little in the literature, however, concerning the incidence and distribution of the disease. The TB MED 208 (44) states that malaria presents a serious problem in the southern part of the peninsula but that it is less prevalent in the northern provinces. Similar information of an equally scanty nature is also given by Boyd (42) and Simmons et al (45). In speaking of the distribution of malaria in Korea, Boyd (42) says that the most conspicuous feature is the relatively light endemicity in Seoul as contrasted with severely endemic foci in southern areas. TB MED 208 (44) gives an official report of 1,123 deaths from malaria in 1938. It is doubtful whether or not such reports are very accurate.

Species of Malaria Present - Recent information indicates that Plasmodium vivax is the dominant parasite (46, 47, 48). Kobayashi (43) reports that this species is found all over Korea, but that in general it is endemic in the low, damp areas of middle Korea, especially Inchon, the Taedong River near Pyongyang, and around Wonsan. Plasmodium malariae was reported from Seoul (43) but is apparently rare. According to the same author (43) there have been only about 20 cases of P. falciparum reported. There was no evidence that these were mosquito-borne, as most were reported from drug addicts who were apparently infected by means of an improperly sterilized needle. It therefore appears that P. vivax is common and the other species rare or non-existent in nature. (49)

The principal mosquito vector of P. vivax is reported to be Anopheles hyrcanus sinensis. It breeds in stagnant pools, streams, irrigation ditches and rice paddies, and is quite widely distributed. Aside from a few small projects in the vicinity of some of the larger cities, no significant work on control of mosquitoes has been carried out (45). Experimentally A. hyrcanus sinensis is a poor vector for P. falciparum (43).

The Situation in 1950 - With the advent of hostilities in Korea the Medical Department was confronted with the problem of malaria control among the Armed Forces. A program of suppressive treatment was initiated as soon as possible. Chloroquine diphosphate, 0.5 gm. (equivalent to 0.3 gm. of the base) was used suppressively, being administered once a week. However, regardless of this and other malaria discipline, cases of malaria had begun to appear by the middle of August. An effort was made by this laboratory to obtain slides on all suspected cases among the patients who were returned to Japan for hospitalization. At the same time additional information was sought concerning the unit, location in Korea, effects of chloroquine suppressive treatment, and other data. This was done by the use of a questionnaire which was distributed to the various hospitals to be returned to this laboratory along with the suspected malarial smear. Unfortunately, some slides were received before the questionnaires were available and in other instances the information was incomplete. Attempts are being made to secure more specific information in an effort to better evaluate the effectiveness of chloroquine suppression and also to delineate the current areas of highest malarial endemicity in Korea.

Discussion of Findings - A total of 378 slides were received from the hospitals. Of these 192 were positive for P. vivax, 183 negative, and 3 were broken in transit (Table XXX). The correlation between the diagnoses of this laboratory and those of the submitting hospitals was good. A summary of these results is given in Table XXXI. The location in Korea was given in only 38 of the 192 positive cases. Twenty-eight of these appeared to have been contracted in the vicinity of Taegu. The remaining ten cases from areas extending south to Pusan and west to Chinju. Several smears were received from patients who had been stationed in the Seoul area but these were all negative. Among the 192 positive cases there were 56 instances in which the parent unit was given. These are listed in Table XXXII.

Table XXX. Malaria Smears Received by 406th Medical General Laboratory During 1950 for Confirmation

Month	Total No. Received	No. Positive	No. Negative	No. Unsatisfactory
August	17	9	8	
September	234	150	84	
October	82	30	52	
November	11	0	11	
December	34	3	28	3 broken
Totals	378	192	183	3

Table XXXI. Summary of Diagnoses of Malaria Slides Received
During 1950 from Korea

No information on original diagnosis	119
Confirmed by 406th Med Gen Lab	247
Submitting hospital diagnosis positive 406th Med Gen Lab diagnosis negative	4
Submitting hospital diagnosis negative 406th Med Gen Lab diagnosis positive	5

Table XXXII. Summary of Malaria Cases by Units of the
Armed Forces During 1950

<u>Unit</u>	<u>No. of Cases</u>
1st Cavalry Division	22
24th Infantry Division	19
25th Infantry Division	11
2nd Infantry Division	4
Division Unknown	136
Total	

The data acquired on the effects of chloroquine suppressive treatment are too limited for an adequate evaluation.

OPTIMUM AMOUNT OF STOOL FOR THE AMS III TECHNIQUE: In the development of the AMS III technique (27), two grams of feces were decided upon arbitrarily as the optimum amount to be used for the test. This quantity gave very satisfactory results on the recovery of schistosome eggs. Since this technique is being utilized routinely for the recovery of all types of helminths eggs it seemed advisable to determine the optimum number of grams of feces for general use.

Enough feces was comminuted in water so that after straining and centrifuging 10 ml. of the mixture contained 2 ml. of sediment. After this standardization further dilutions were made to include 1.5 ml., 1.0 ml., 0.5 ml. and 0.25 ml. Two tubes of each dilution for each specimen were used in the comparison and the routine AMS III technique was carried out. After the final supernate was discarded the concentrate was diluted so that the smears would be relatively uniform in density. Accurate egg counts were made and the ease of examining the slide was noted. Twenty different stool specimens were studied in this manner. Since no definite end point was established with this amount, it was decided to run more specimens with an increased amount of stool. Thirteen additional stools were examined comparing 2.0 ml. and 3.0 ml. of sediment.

The data indicate that 1 ml. to 2 ml. of fecal sediment is satisfactory for recovery of all helminth eggs with the AMS III technique. Three ml. did, however, give an increased recovery. The recovery of *S. japonicum* eggs was relatively uniform from 0.5 ml. to 2.0 ml., but then showed an increase with the larger quantity.

Less than 1.0 ml. of sediment is definitely undesirable for all types of eggs.

Although 3.0 ml. is still not the end point, larger amounts are not practical because of difficulty in carrying out the technique with the heavy sediment.

Summary - Good results can be obtained with the AMS III technique using amounts of fecal sediment ranging from 1.0 ml. to 3.0 ml. in a 15.0 ml. centrifuge tube. Although the egg count continues to go up for amounts over 2.0 ml., this amount appears to be optimum, as the advantages of increased recovery of all helminth eggs is noticeably offset by mechanical difficulties in preparing and examining the specimen.

A COMPARISON OF TRITON-NE WITH QM LAUNDRY DETERGENT IN THE AMS III STOOL CONCENTRATION TECHNIQUE: The addition of Triton-NE to various acid-ether concentration techniques for the recovery of helminth eggs has been used successfully since 1945. The AMS III technique using Triton-NE has been found to be excellent for the recovery of eggs of Schistosoma japonicum as well as other helminths. Muschel (28) reported in 1949 that he has good results in recovering schistosome eggs by substituting a 10% aqueous solution of mobile laundry detergent (QM Item 51-D-175) for the Triton-NE. Since the AMS III is used routinely in our epidemiologic surveys, it was decided to run a series of comparative tests between the two (29).

The results secured on the same stool samples using both detergents are summarized in Table XXXIII. A total of 61 fresh and 15 preserved stools were tested. Forty-nine of the fresh stools yielded more eggs of S. japonicum with Triton-NE, while only two gave better results with the QM detergent. There were a total of 309 schistosome eggs recovered when Triton-NE was used and only 49 when the laundry detergent was substituted. In the case of Ascaris lumbricoides, Trichuris trichiura and hookworm, the use of Triton-NE resulted in more eggs being recovered in 71% to 95% of the cases. Furthermore, the total number of eggs recovered definitely favored the use of Triton-NE. Only in the case of Trichostrongylus sp. were the Triton-NE and QM detergent nearly equal.

Fifteen preserved stools were also tested. In only one instance did the QM detergent recover more eggs than the Triton-NE. In all other cases the total number of eggs recovered was markedly higher when Triton-NE was utilized (Table XXXIII).

Table XXXIII. Comparison of the Results Obtained Using Triton-NE and QM Laundry Detergent

Helminths	TESTS ON 61 FRESH STOOLS				
	No. of Times Triton-NE Recovered More Eggs	No. of Times Laundry Detergent Recovered More Eggs	No. of Times Same Recovery By Both Techniques	Total No. Eggs Recovered With Triton-NE	Total No. Eggs Recovered with Laundry Detergent
<u>S. japonicum</u>	49	2	3	309	49
<u>Ascaris</u>	38	11	0	1,354	530
<u>Trichuris trichiura</u>	55	4	2	2,610	1,863
<u>Hookworm</u>	36	2	0	238	42
<u>Trichostrongylus</u> sp.	4	3	3	7	5
<u>Pinworm</u>	2	0	0	2	0

TESTS ON 15 PRESERVED STOOLS					
<u>S. japonicum</u>	12	0	0	37	5
<u>Ascaris</u>	15	0	0	1,001	113
<u>Trichuris trichiura</u>	15	0	0	338	42
<u>Hookworm</u>	12	1	0	63	6
<u>Trichostrongylus</u> sp.	3	0	0	3	0
<u>Clonorchis sinensis</u>	1	0	0	160	69
<u>Paragonimus westermani</u>	1	0	0	4	1

Summary - The results obtained in the comparison of Triton-NE with QM Laundry detergent (#51-D-175) clearly demonstrate the superiority of the Triton-NE in the recovery of the eggs of S. japonicum, A. lumbricoides, T. trichiura and hookworm. It is better for the eggs of Trichostrongylus sp., C. sinensis, P. westermani as well as the others mentioned above when preserved stools are tested.

EVALUATION OF FROG PREGNANCY TESTS: Introduction - The use of amphibia as test animals for pregnancy was begun in 1934 by Shapiro and Zwarenstein (30) using the South African clawed frog, Xenopus laevis. In 1947, Galli-Mainini (31) reported satisfactory results with a native male toad as the test animal in Argentina. Wiltberger and Miller (1948) in the United States found that the male Leopard frog, Rana pipiens, was also suitable for use in pregnancy determinations (32). These tests are simple, rapid, require no dissection to determine results and so eliminate subjective interpretations which are often required in the Friedman test (33).

Methods - Briefly the technique consists of the injection of a few ml. of the patient's urine, previously concentrated (34) into the dorsal lymph sac of the amphibian. Positive results are indicated by the extrusion of eggs, usually within eight to thirty hours, in the case of female Xenopus laevis, and the deposition of spermatozoa in the cloaca of the male amphibian. In the case of the latter, a drop of urine is recovered from the animal with a capillary pipette each hour for four hours after injection and examined under the microscope for the presence of spermatozoa.

Evaluation of Results - Since November, 1948 the South African clawed frog, X. laevis, has been used in this laboratory as a test animal. This species must be imported and is relatively expensive. Therefore, it was decided to seek a cheaper, more readily available substitute among the indigenous amphibia. The Japanese species, Rana nigromaculata, appeared suitable as a possible replacement for X. laevis. With this in mind specimens were procured locally and a series of parallel tests was begun with X. laevis in order to determine its relative efficiency as a test animal. Robbins et al (35) found in a study of 100 cases of pregnancy or suspected pregnancy that X. laevis determined correctly 96% of the cases, hence this frog was used as the base line in the present evaluation. Out of 149 duplicate tests R. nigromaculata yielded 47 positive and 3 negative reactions on 50 urines that were positive by X. laevis and 3 false positives out of 99 urines negative by X. laevis in 1949.

In 1950 the program was further expanded to include the final clinical findings of the patients as a basis for evaluating the efficiency of these two frogs. This approach was considered to be more accurate since it eliminated any errors due to X. laevis. Therefore, whenever possible both amphibia were used in parallel series and the results were correlated with the patients' final clinical findings which were obtained from questionnaires sent to the various hospitals and dispensaries in the Far East Command.

During the year clinical and laboratory findings on 244 patients, each tested by both amphibia, were assembled and the results compared (Table XXXIV). In addition to the above, 205 and 39 patients were tested singly by Rana and Xenopus respectively. These results were also compared with the clinical findings and are reported in Table XXXIV. Differences in the results obtained by the two animals tested in series were not significant by the Chi-Squared test (10). Rana nigromaculata and Xenopus laevis revealed 86.8% and 83.7% respectively of the 129 positive cases. Clinical findings showed that of 115 cases which were negative, R. nigromaculata determined correctly 94.8% and X. laevis 92.2%; the respective false positives being 5.2% and 7.8%. Each test animal produced almost twice as many false negatives as false positives, the results being 13.2% and 16.3% for R. nigromaculata and X. laevis respectively. The greatest inconsistency occurred during March 1950 when eight of 36 that were tested on both animals gave conflicting results. Clinical findings that were obtained for 5 of the inconsistencies showed that X. laevis was correct in three cases and R. nigromaculata in two.

Table XXXIV. Summary of the Results of Laboratory Tests for Pregnancy, Using Xenopus laevis and Rana nigromaculata, and the Clinical Findings on 244 Patients

Test Animal Used	Total Labor- atory Tests	Total Positive By Clinical Findings	Evaluation of Lab- oratory Findings				Total Negative By Clinical Findings	Evaluation of Lab- oratory Findings			
			True		False			True		False	
			Positives	Negatives	Positives	Negatives		Negatives	Positives	Negatives	Positives
			No.	%	No.	%		No.	%	No.	%
<u>X. laevis</u>	244	129	108	83.7	21	16.3	115	106	92.2	9	7.8
<u>R. nigromaculata</u>	244	129	112	86.8	17	13.2	115	109	94.8	6	5.2

Table XXXV. Summary of Laboratory Results Obtained with a Single Species As Compared with the Clinical Findings

Test Animal Used	Total Labor- atory Tests	Total Positive By Clinical Findings	Evaluation of Lab- oratory Findings				Total Negative By Clinical Findings	Evaluation of Lab- oratory Findings			
			True		False			True		False	
			Positives	Negatives	Positives	Negatives		Negatives	Positives		
			No.	%	No.	%		No.	%	No.	%
<u>X. laevis</u>	39	24	16	66.7	8	33.3	15	11	73.3	4	26.7
<u>R. nigromac- ulata</u>	205	148	129	87.2	19	12.8	57	47	82.5	10	17.5

Xenopus laevis was used alone in 39 pregnancy tests, correct determinations being as follows: 66.7% of the 24 positive cases, and 73.3% of the 11 negative ones. The false positives and negatives were 27.7% and 33.3% respectively. This was a small sample and of doubtful significance.

Summary - The results of the study using R. nigromaculata and X. laevis in parallel tests on 244 patients, 129 of which were positive and 115 negative for pregnancy, show that these amphibia are equally efficient as test animals for pregnancy. Differences in results were of no statistical significance. Both animals produced almost twice as many false negatives as false positives. In view of the results obtained, the use of R. nigromaculata for pregnancy determination in Japan instead of X. laevis is considered practical.

DEPARTMENT OF BACTERIOLOGY

The department is composed of the following functional sections:

1. Diagnostic Medical Bacteriology
Bacteriology of Water, Food, and Dairy Products Subsection
Mycology Subsection
2. Enteric Bacteriology
3. T. B. Bacteriology
4. Biologics Production
5. Special Projects
Bioassay Subsection
6. Service
Animal Room
Media Preparation
Central Supply

Routine

A total of 77,005 routine procedures were conducted in examining 31,924 specimens during 1950, representing a 66% increase over that of the preceding year. The marked increase primarily was due to the Korean situation and to intensified investigation of the ubiquitous pathogen of Japan and Korea - the Shigella.

In addition to standard diagnostic support of local dispensaries, and confirmation or definitive identification of suspect cultures from other medical installations, this department is often solely involved with other units of this laboratory in special projects which arise. These duties have included production of pyrogen-free water, studies on bacteriology of war wounds, examination of suspect unknown agents, and others. Assistance in special epidemiologic problems have also included 4 food poisoning outbreaks, a survey for diphtheria, a shipboard dysentery epidemic, and surveys of veterans of the Korean war.

PRODUCTION OF PYROGEN-FREE WATER: With the establishment of a blood bank in the laboratory this department was requested to produce sufficient pyrogen-free water for the re-processing of rubber tubing, needles, etc. This was required since limited stocks of commercially prepared product were destined for essential field use. Adequate equipment necessary for large scale production was not available and it was therefore necessary to resort to the re-distillation of small amounts of treated distilled water.

The method used by us principally follows that described in the Remington's "Practice of Pharmacy" (1). Eight hundred ml. of distilled water is delivered in a distillation flask (1000 ml.) connected to a Graham condenser which is attached with a glass shield. To the above 800 ml. distilled water, 1.6 ml. of 0.5 N potassium permanganate and 4 ml. of 43 grams per liter solution of sodium hydroxide are added. Several glass beads are put in to prevent violent boiling. The distilled water is gently boiled and the distillate is collected in a tall 50 ml. Nessler tube. As soon as the Nessler tube is filled to the 50 ml. mark, 2 ml. of alkaline mercuric potassium iodide solution (Nessler's reagent) is added. When the distillate tested in this manner shows no yellowish discoloration (no oxidizable substances present) the collecting tube is replaced by a pyrogen-free vacoliter bottle and the distillation is continued. When approximately one-tenth of the original distilled water remains in the distillation flask, the collection of distillate shall cease. The collected re-distilled water is autoclaved as soon as possible at 15 pounds for 15 minutes, three times, with 4 hour intervals. Employing this technique three hours are required to produce 1000 ml. of re-distillate. Seven sets of condenser apparatus were set up, and when operated 24 hours each day, allowed a daily capacity of approximately 50 liters. Water from each of the 7 sets was

pyrogen tested in rabbits and found acceptable according to the provisions set forth in the USP (1st Supplement) 12th Revision, p. 606-607 (2). Likewise specimens from each of the two stills were examined for total solids and heavy metals by the Chemistry Department; each was acceptable in accordance with the requirements of USP for re-distilled water.

Many problems developed, the most serious being the procurement of Graham flasks and condensers. Initially only 7 such apparatus were available and, although manufactured of pyrex glass, were not capable of withstanding continued heating. With the aids of indigenous glass blowers it was possible to repair broken flasks rapidly enough to produce approximately 50 liters in each 24 hour period of operation.

The period of our operation was from 13 July to 11 October 1950, when a commercially prepared product from the ZI became available. In this period 1,249 one liter bottles were delivered to the blood bank. Approximately 50 one liter bottles were used for control purposes such as the determination of total solids and the testing for pyrogens in rabbits.

ANTISTREPTOLYSIN TITRES OF 1207 "NORMAL" CARDIOLIPIN NEGATIVE SERA COLLECTED IN 1950: The ease with which local clinicians appeared satisfied with single determinations of antistreptolysins stimulated preliminary investigation of source material to determine if any arbitrary level of this antibody had acceptable significance. Negative results suggested our own investigation to determine what could be considered "normal" for U. S. personnel in this area. Twelve hundred seven serum samples, cardiolipin negative, were secured at random from Serology Department during the year in an effort to determine an antistreptolysin "normal" titer. Results are summarized in the following table.

Table I

<u>Titer (units/ml.)</u>	<u>Frequency</u>	<u>Percentage</u>
10	13	1.1%
20	44	3.6%
40	149	12.3%
80	345	28.5%
160	438	36.2%
320	184	15.5%
640	32	2.7%
1280	2	0.2%
Total	1207	100.0%

It is noticed that the maximum incidence of distribution of "normal" antistreptolysin titers falls in 160 units per ml. of serum during the year. Season variation of distribution of "normal" antistreptolysin titer: The distribution of normal antistreptolysin titer for each month are shown in Tables II, III, IV, and V.

It is noticeable that the mode of distribution of "normal" antistreptolysin for January through September falls in 160 units class whereas that for October through December falls in the 80 units class. As a conclusion it may be stated that:

1. About 40 per cent of cardiolipin negative sera contains 160 units of antistreptolysin per ml.
2. About 30 per cent of cardiolipin negative sera contains 80 units of antistreptolysin per ml.
3. It is rare that cardiolipin negative serum contains more than 1000 units of antistreptolysin per ml.
4. At the outset of the winter season antistreptolysin titer in cardiolipin negative serum is lower than that in other seasons of the year with late summer indicating a peak in that 51% of all sera falls in the 160 units class in August.

Table II

Titer (units/ml.)	January		February		March	
	Freq.	%	Freq.	%	Freq.	%
10	1	1.2	2	3.3	1	2.0
20	0	0	2	3.3	1	2.0
40	4	4.9	7	11.6	1	2.0
80	21	26.0	16	26.6	13	26.0
160	33	40.8	20	33.3	23	46.0
320	20	24.6	12	20.0	11	22.0
640	2	2.5	1	1.7	0	0
1280	0	0	0	0	0	0
Total	81	100.0	60	100.0	50	100.0

Table III

Titer (units/ml.)	April		May		June	
	Freq.	%	Freq.	%	Freq.	%
10	0	0	0	0	4	3.3
20	6	3.6	0	0	5	4.2
40	18	10.8	10	10.0	10	8.3
80	48	28.8	19	19.0	34	28.3
160	59	35.5	45	45.0	44	36.7
320	25	15.0	19	19.0	12	15.0
640	9	5.4	7	7.0	5	4.2
1280	1	0.6	0	0	0	0
Total	166	100.0	100	100.0	120	100.0

Table IV

Titer (units/ml.)	July		August		September	
	Freq.	%	Freq.	%	Freq.	%
10	0	0	0	0	0	0
20	3	3.3	1	1.0	5	4.5
40	9	10.0	11	11.0	15	13.6
80	21	23.3	26	26.0	27	24.6
160	41	45.6	51	51.0	37	33.6
320	16	17.8	9	9.0	22	20.0
640	0	0	2	2.0	3	2.7
1280	0	0	0	0	1	0.9
Total	90	100.0	100	100.0	110	100.0

Table V

Titer (units/ml.)	October		November		December	
	Freq.	%	Freq.	%	Freq.	%
10	3	2.3	1	1.0	1	1.0
20	11	8.5	6	6.0	4	4.0
40	23	17.7	20	20.0	21	21.0
80	50	38.5	39	39.0	31	31.0
160	36	27.6	21	21.0	28	28.0
320	7	5.4	12	12.0	13	13.0
640	0	0	1	1.0	2	2.0
1280	0	0	0	0	0	0
Total	130	100.0	100	100.0	100	100.0

EFFECT OF CHLORINE ON LEPTOSPIRA IN WATER: Proven presence of Leptospira in field rodents trapped in maneuver areas gave rise to the question of whether or not usual field water purification methods would be sufficient for surface waters contaminated with such organisms. An in vitro experiment was performed as follows:

Leptospira icterohemorrhagiae was grown in Korthof's media for 4 days at 37°C. The culture was centrifuged to be concentrated to 1/12 volume, 0.2 ml. of which was inoculated in 2.2 ml. of tap-water containing varying amount of chlorine, the concentration of Leptospira thus being put back to the original culture. After certain periods of time as specified in the following table, 0.2 ml. of each chlorinated leptospira-suspension in water was inoculated in 2.2 ml. Korthof's media to determine the viability of the Leptospira exposed to the water containing varying amount of free chlorine as indicated in the following table.

Table VI. Viability of Leptospira in Chlorinated Water

Exposure Period	Amount of chlorine (p.p.m.)									Growth Control
	5.0	4.0	3.0	2.0	1.0	0.8	0.4	0.2	0.1	
15'	-	-	-	/	/	/	/	/	/	/
30'	-	-	-	-	-	-	-	/	/	/
60'	-	-	-	-	-	-	-	/	/	/
90'	-	-	-	-	-	-	-	/	/	/
120'	-	-	-	-	-	-	-	/	/	/
180'	-	-	-	-	-	-	-	/	/	/

/ Indicates positive subculture

- Indicates negative subculture

From the above results it is shown that 3 p.p.m. of chlorine in tap water can kill the Leptospira icterohemorrhagiae under the above conditions in 15 minutes, 0.4 p.p.m. of chlorine in 30 minutes, and 0.2 p.p.m. is not effective even after a 3 hours period.

The same experiment was carried out in chlorinated Korthof's media, the results of which shows that as high as 5 p.p.m. of chlorine in Korthof's media is not sufficient concentration to kill the Leptospira.

MEDIUM FOR THE ISOLATION OF HEMOPHILUS DUCREYI: Numerous attempts to isolate Hemophilus ducreyi, employing various culture media, have been made in this department in recent years without success. Early in 1950, we succeeded in growing the organism in a serum enriched (20%) tryptose phosphate broth but all attempts to subculture the organism, in pure culture, to a solid medium failed.

Following experimentation with several solid media we devised a formula which meets the growth requirements of Hemophilus ducreyi and, when working with pure cultures, experienced no difficulty in culturing the organism. The formula is as follows:

Proteose peptone #3	20.0 gm.
Sodium chloride	5.0 gm.
Disodium phosphate	2.5 gm.
Dextrose	1.0 gm.
Agar	18.0 gm.
Distilled water	1,000.0 ml.

Adjust pH to 7.6 and autoclave at 15 lbs. for fifteen minutes. Cool to 45°C. and add blood (human or rabbit) and yeast extract to a final concentration of 15% and 1% respectively. Pour in petri dishes for use.

The second phase of this work was an attempt to isolate Hemophilus ducreyi directly from suspect penile lesions. Here the problem has been the faster growing strains of concomitant bacteria such as Staphylococcus, Streptococcus and the Gram-negative flora. The fastidious nature of Hemophilus ducreyi precluded employment of the usual inhibitory dyes and chemicals. It was thought reasonable, therefore, to search for an antibiotic which may be ineffective for Hemophilus ducreyi but effective against the concomitant flora. Since the sulfonamide compounds are generally considered specific in the treatment of chancroid disease the sensitivity tests were done only with penicillin, streptomycin, aureomycin and chloromycetin. The minimal concentration of the various antibiotics effective against Hemophilus ducreyi is shown in Figure 1. As indicated the concentrations effective against Hemophilus ducreyi are appreciably lower than those required for the inhibition of usual concomitant flora.

Considering the accepted clinical observation of inadvisability of penicillin therapy in pertussis, and possible enhancement of the growth of Hemophilus pertussis it was considered worthwhile to attempt to learn whether that was also true for Hemophilus ducreyi. Employing penicillin, streptomycin and chloromycetin, 0.03 units/ml., 0.3 units/ml. and 0.05 mcg/ml. respectively, enhancement of growth was effected as shown in Table VII.

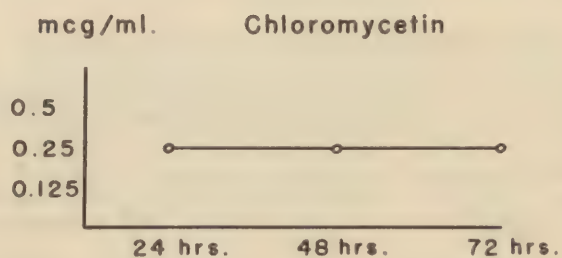
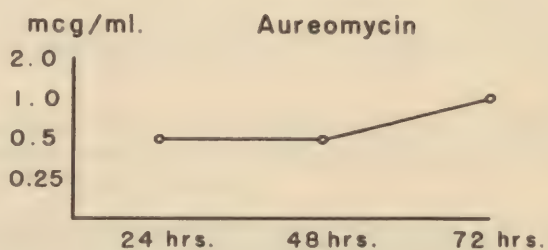
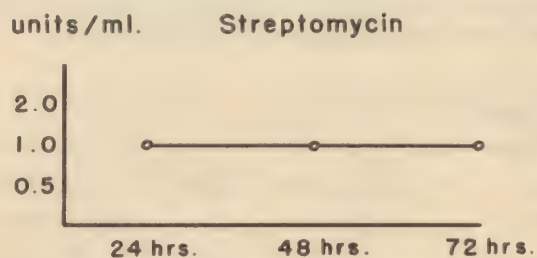
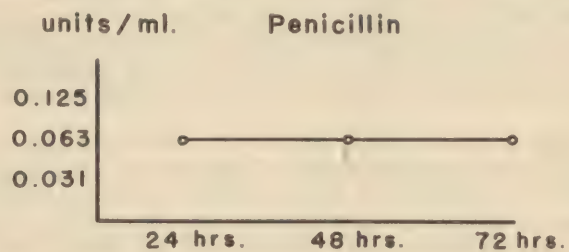
Table VII. Number of Colonies of Hemophilus ducreyi on Blood Agar Plate Containing Various Antibiotics

Medium	No. of plate					Average
	1	2	3	4	5	
Penicillin plate (0.03 units/ml.)	300	400	200	160	200	252
Streptomycin plate (0.3 units/ml.)	1200	1400	1600	1600	800	1320
Chloromycetin plate (0.05 mcg/ml.)	3000	3000	3200	3200	3000	3080
Control plate without antibiotic	200	220	300	200	150	214

As indicated in Table VII, 0.05 mcg/ml. of chloromycetin in yeast enriched blood agar greatly enhances the growth of Hemophilus ducreyi. Although these studies were carried out employing stock Lederle strains we have, employing this method, successfully isolated the organism directly from a penile lesion.

ANTIBIOTIC SENSITIVITIES OF FRESHLY ISOLATED SHIGELLA STRAINS: Some doubt appears to exist concerning efficacy of antibiotics in bacillary dysentery (O) in comparison with sulfonamides. In view of the military importance of such knowledge, and considering that in vitro the agar-cap method was employed, readings being recorded after 20 hours of incubation at 37°C. All freshly isolated organisms were tested within 72 hours following initial streaking for identification. Fifty-nine strains including members of Shigella Groups A, B, and C were tested against chloromycetin, aureomycin, streptomycin, penicillin, sulfadiazine, sulfaguanidine, and terramycin.

The results are shown in Tables VIII, IX, X, XI, XII, XIII, XIV. As is indicated therein, all Shigella strains with the exception of sonnei are markedly sensitive to chloromycetin and terramycin, and it is apparent that aureomycin and streptomycin effect some inhibition against freshly isolated strains. Penicillin, sulfadiazine and sulfaguanidine appear to be ineffective against all strains tested in vitro.



Minimal Inhibitory Amount of Antibiotic
Against H. ducreyi

Figure 1.

Table VIII. Sensitivities of Shigella to Chloromycetin

<u>Organism</u>	<u>No.</u>	<u>Minimal inhibitory concentration</u>
1. <u>Sh. dysenteriae</u>	5	2.5 - 5.0 mcg/ml.
2. <u>Sh. para-W</u>	17	2.5 - 5.0 mcg/ml.
3. <u>Sh. para-X</u>	2	5.0 mcg/ml.
4. <u>Sh. para-Z</u>	8	2.5 - 10 mcg/ml.
5. <u>Sh. para-WX</u>	2	5.0 mcg/ml.
6. <u>Sh. para-VZ</u>	2	5.0 mcg/ml.
7. <u>Sh. schmitzi</u> (<u>ambigua</u>)	1	2.5 mcg/ml.
8. <u>Sh. alkalescens</u>	1	5.0 mcg/ml.
9. <u>Sh. boyd-88</u>	2	2.5 mcg/ml.
10. <u>Sh. boyd-103</u>	2	5.0 mcg/ml.
11. <u>Sh. boyd-170</u>	2	5.0 mcg/ml.
12. <u>Sh. P-119</u>	1	2.5 mcg/ml.
13. <u>Sh. sonnei</u>	5	10 - 40 mcg/ml.
14. Paracolon	1	20 mcg/ml.
Total	41	

Table II. Sensitivities of Shigella to Aureomycin

<u>Organism</u>	<u>No.</u>	<u>Minimal inhibitory concentration</u>
1. <u>Sh. dysenteriae</u>	5	10 - 80 mcg/ml.
2. <u>Sh. para-W</u>	9	5 - 40 mcg/ml.
3. <u>Sh. para X</u>	2	20 mcg/ml.
4. <u>Sh. para-Z</u>	6	10 - 40 mcg/ml.
5. <u>Sh. para-WX</u>	1	20 mcg/ml.
6. <u>Sh. para-VZ</u>	1	40 mcg/ml.
7. <u>Sh. schmitzi</u>	1	10 mcg/ml.
8. <u>Sh. alkalescens</u>	1	100 mcg/ml.
9. <u>Sh. boyd-88</u>	1	20 mcg/ml.
10. <u>Sh. boyd-103</u>	1	20 mcg/ml.
11. <u>Sh. boyd-170</u>	1	20 mcg/ml.
12. <u>Sh. P-119</u>	1	20 mcg/ml.
13. <u>Sh. sonnei</u>	5	40 - 80 mcg/ml.
14. Paracolon	1	100 mcg/ml.
	36	

Table X. Sensitivities of Shigella to Streptomycin

<u>Organism</u>	<u>No.</u>	<u>Minimal inhibitory concentration</u>
1. <u>Sh. dysenteriae</u>	5	5 - 20 units/ml.
2. <u>Sh. para-W</u>	9	2.5 - 10 units/ml.
3. <u>Sh. para-X</u>	2	10 units/ml.
4. <u>Sh. para-Z</u>	6	5 - 10 units/ml.
5. <u>Sh. para-WX</u>	1	10 units/ml.
6. <u>Sh. para-VZ</u>	1	5 units/ml.
7. <u>Sh. schmitzi</u>	1	10 units/ml.
8. <u>Sh. alkalescens</u>	1	20 units/ml.
9. <u>Sh. boyd-88</u>	1	5 units/ml.
10. <u>Sh. boyd-103</u>	1	10 units/ml.
11. <u>Sh. boyd 170</u>	1	10 units/ml.
12. <u>Sh. P-119</u>	1	10 units/ml.
13. <u>Sh. sonnei</u>	5	5 - 40 units/ml.
14. Paracolon	1	5 units/ml.
Total	36	

Table XI. Sensitivities of Shigella to Penicillin

<u>Organism</u>	<u>No.</u>	<u>Minimal inhibitory concentration</u>
1. <u>Sh. dysenteriae</u>	5	10 - 80 units/ml.
2. <u>Sh. para-W</u>	9	40 - 80 units/ml.
3. <u>Sh. para-X</u>	2	40 units/ml.
4. <u>Sh. para-Z</u>	6	40 - 80 units/ml.
5. <u>Sh. para-WX</u>	1	40 units/ml.
6. <u>Sh. para-VZ</u>	1	40 units/ml.
7. <u>Sh. schmitzi</u>	1	40 units/ml.
8. <u>Sh. alkalescens</u>	1	160 units/ml.
9. <u>Sh. boyd-88</u>	1	40 units/ml.
10. <u>Sh. boyd-103</u>	1	80 units/ml.
11. <u>Sh. boyd-170</u>	1	80 units/ml.
12. <u>Sh. P-119</u>	1	80 units ml.
13. <u>Sh. sonnei</u>	5	40 - 80 units/ml.
14. Paracolon	1	80 units/ml.
	36	

Table XII. Sensitivities of Shigella to Sulfadiazine

<u>Organism</u>	<u>No.</u>	<u>Minimal inhibitory concentration</u>
1. <u>Sh. dysenteriae</u>	5	5 - 20 mg/ml.
2. <u>Sh. para-W</u>	9	20 - >20 mg/ml.
3. <u>Sh. para-X</u>	2	>20 mg/ml.
4. <u>Sh. para-Z</u>	6	20 - >20 mg/ml.
5. <u>Sh. para-WX</u>	1	>20 mg/ml.
6. <u>Sh. para-VZ</u>	1	20 mg/ml.
7. <u>Sh. schmitzi</u>	1	>20 mg/ml.
8. <u>Sh. alkalescens</u>	1	>20 mg/ml.
9. <u>Sh. boyd-88</u>	1	>20 mg/ml.
10. <u>Sh. boyd-103</u>	1	20 mg/ml.
11. <u>Sh. boyd-170</u>	1	20 mg/ml.
12. <u>Sh. P-119</u>	1	20 mg/ml.
13. <u>Sh. sonnei</u>	5	>20 mg/ml.
14. Paracolon	1	>20 mg/ml.
Total	36	

Table XIII. Sensitivities of Shigella to Sulfaguanidine

<u>Organism</u>	<u>No.</u>	<u>Minimal inhibitory concentration</u>
1. <u>Sh. dysenteriae</u>	5	2.5 - 5 mg/ml.
2. <u>Sh. para-W</u>	9	5 - >5 mg/ml.
3. <u>Sh. para-X</u>	2	5 mg/ml.
4. <u>Sh. para-Z</u>	6	5 - >5 mg/ml.
5. <u>Sh. para-WX</u>	1	>5 mg/ml.
6. <u>Sh. para-VZ</u>	1	5 mg/ml.
7. <u>Sh. schmitzi</u>	1	5 mg/ml.
8. <u>Sh. alkalescens</u>	1	>5 mg/ml.
9. <u>Sh. boyd-88</u>	1	>5 mg/ml.
10. <u>Sh. boyd-103</u>	1	5 mg/ml.
11. <u>Sh. boyd-170</u>	1	5 mg/ml.
12. <u>Sh. P-119</u>	1	5 mg/ml.
13. <u>Sh. sonnei</u>	5	>5 mg/ml.
14. Paracolon	1	>5 mg/ml.
Total	36	

Table XIV. Sensitivities of Shigella to Terramycin

<u>Organism</u>	<u>No.</u>	<u>Minimal inhibitory concentration</u>
1. <u>Sh. dysenteriae</u>	2	.5 - 10 mcg/ml.
2. <u>Sh. para-W</u>	4	2.5 - 5 mcg/ml.
3. <u>Sh. para-X</u>	1	1.25 mcg/ml.
4. <u>Sh. para-Z</u>	6	1.25 - 5 mcg/ml.
5. <u>Sh. para-WX</u>	1	1.25 mcg/ml.
6. <u>Sh. para-VZ</u>	1	5 mcg/ml.
7. <u>Sh. boyd-103</u>	2	5 - 10 mcg/ml.
8. <u>Sh. sonnei</u>	1	20 mcg/ml.
Total	18	

SURVEY OF ENTERIC PATHOGENS IN THE JAPANESE, TOKYO, 1950: Commencing 1 March 1950, plans were formulated for an extensive enteric survey in conjunction with the Japanese National Institute of Health. The initial considerations were standardization of methods and nomenclature. Six Japanese hospitals cooperated in the survey under the supervision of the National Institute of Health. They were: Honjo, Komagome, Toshima, Toyotama, Ebara, and Denken Hospital.

Comparison of stock strains in our files with those of the National Institute of Health was first performed to attempt some standardization of nomenclature. As part of this step, 13 cultures representing the standard Shigella strains encountered in Japan were submitted to us. The biochemical activity and serological reactions against our serum is shown in Tables XV and XVI. Comparative nomenclature based on these findings is shown in Table XVII. As is indicated therein the Nakamura and Sueishi strains differ only in that the latter produces indol; Saigon-Arai and Sigon-18 are serologically identical.

Table XV. Biochemical Reactions Shigellae - Japanese Classification

<u>Strain</u>	<u>Dextrose</u>	<u>Trehalose</u>	<u>Maltose</u>	<u>Sucrose</u>	<u>Lactose</u>	<u>Raffinose</u>	<u>Arabinose</u>	<u>Xylose</u>	<u>Rhamnose</u>	<u>Mannite</u>	<u>Dulcitol</u>	<u>Inositol</u>	<u>Sorbitol</u>	<u>Dextrin</u>	<u>Glycerol</u>	<u>Gas</u>	<u>Citrate</u>	<u>Indol</u>	<u>H₂S</u>	<u>Urease</u>	<u>Motility</u>	<u>Salicin</u>
Shiga	1*	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ohno	1	7	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	+	-	-	-	-
Nakamura	1	1	2	-	-	-	2	-	-	1	-	-	-	2	8	-	-	-	-	-	-	-
Komagome-B III	1	1	-	-	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Komagome-A	1	1	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Kawase	1	2	-	-	-	-	2	-	8	1	-	-	1	-	-	-	-	-	-	-	-	-
Saigon-18	1	-	1	-	-	-	1	-	-	-	-	-	4	2	-	-	-	+	-	-	-	-
Saigon-Arai	1	-	1	-	-	-	1	8	-	-	-	-	2	2	-	-	-	+	-	-	-	-
Komagome-B I	1	1	1	-	-	-	2	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
Showa	1	1	-	8	-	2	4	-	-	1	-	-	-	-	7	-	-	-	-	-	-	-
Mita	1	2	7	-	-	-	4	-	-	1	-	-	-	-	4	+	-	-	-	-	-	-
Sueishi	1	1	1	-	-	8	2	-	-	1	-	-	-	2	8	-	-	+	-	-	-	-
Ohara	1	1	2	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-

* Numeral indicates days required to ferment carbohydrate.

The usual cultures were made in each of the 6 participating hospitals daily and in addition material was inoculated into 2 tubes Selenite F holding medium, one each on clinically suspect stool specimens for the 406th and NIH laboratories. The hospitals inoculated isolation media directly from the fresh stool and forwarded all suspect cultures to NIH for serological classification. Serum employed in our part of the survey was prepared in this laboratory.

The collection of samples commenced on 3 March 1950, and continued to 24 June 1950. Predominate isolations by monthly periods are given in Tables XVIII, XIX, XX, and XXI. Predominate species isolated by monthly periods are given in Table XXII.

Table XVI. Serological Reactions Shigellae - Japanese Classification

Serum		dysen.	ambigua	V	W	Z	103	P-119	88	sonnei
Strain				I	II	III	IV	V	VI	
Shiga	/	-	-	-	-	-	-	-	-	-
Ohno	-	/	-	-	-	-	-	-	-	-
Nakamura	-	-	/	-	-	-	-	-	-	-
Komagome-B III	-	-	-	/	-	-	-	-	-	-
Komagome-A	-	-	-	-	-	-	-	/	-	-
Kawase	-	-	-	-	-	/	-	-	-	-
Saigon-18	-	-	-	-	-	-	/	-	-	-
Komagome-B I	-	-	-	-	-	-	-	-	-	-
Showa	-	-	-	/	-	/	-	-	-	-
Mita	-	-	-	-	-	-	-	-	/	-
Sueishi	-	-	-	/	-	-	-	-	-	-
Ohara	-	-	-	-	-	-	-	-	-	/

Table XVII. Identification by 406th MGL Shigellae- Comparative Nomenclature

<u>Japanese N.I.H.</u>	<u>406th MGL</u>
Shiga	<u>Shigella dysenteriae</u>
Ohno	<u>Shigella ambigua</u>
Nakamura	<u>Shigella paradysen.</u> I (V)
Komagome-B III	<u>Shigella paradysen.</u> II (W)
Komagome-A	<u>Shigella paradysen.</u> V (P-119)
Kawase	<u>Shigella paradysen</u>
Saigon-18	<u>Shigella paradysen.</u> IV (103)
Saigon-Arai	<u>Shigella paradysen.</u> IV (103)
Komagome-B I	<u>Shigella paradysen.</u> VIII (Y)
Showa	<u>Shigella paradysen.</u> I, III (VZ)
Mita	<u>Shigella paradysen.</u> VI (88) Manchester type
Sueishi	<u>Shigella paradysen.</u> I (V)
Ohara	<u>Shigella sonnei</u> I

Table XVIII. Results on Clinical Cases

3 Mch 1950 to 31 Mch	Honjo	Komagome	Toshima	Toyotama	Ebara	Denken	Total
NEP*	22	20	23	32	37	14	148
<u>Sh. paradysen.</u> VZ	0	1	0	0	1	0	2
<u>Sh. paradysen.</u> W	2	4	1	6	2	0	15
<u>Sh. paradysen.</u> WX	0	2	1	2	1	0	6
<u>Sh. paradysen.</u> Z	1	2	0	0	1	0	4
<u>Sh. paradysen.</u> Y	1	0	0	0	0	0	1
<u>Sh. sonnei</u> I	3	0	0	0	0	0	3
<u>Sh. alkalescens</u> I	1	0	0	0	0	0	1
<u>S. typhosa</u>	0	11	5	0	5	0	21
<u>S. paratyphi</u> A	0	2	0	0	3	0	5
Total of the month	30	42	30	40	50	14	206

Table XIX. Results on Clinical Cases

1 Apr 1950 to 30 Apr	Honjo	Komagome	Toshima	Toyotama	Ebara	Denken	Total
NEP*	13	15	18	36	29	1	112
<u>Sh. paradyesen.</u> VZ	0	0	0	0	1	0	1
<u>Sh. paradyesen.</u> W.	1	4	2	3	3	0	13
<u>Sh. paradyesen.</u> WX	0	2	1	2	3	0	8
<u>Sh. paradyesen.</u> Boyd-103	1	0	0	0	0	0	1
<u>Sh. paradyesen.</u> Y	2	0	0	0	0	0	2
<u>Sh. sonnei</u> I	0	0	0	0	3	2	5
<u>S. typhosa</u>	1	1	1	1	0	0	4
<u>S. paratyphi</u> A	0	0	0	3	0	0	3
<u>Paracolobactrum</u> inter-medium	0	0	0	0	1	0	1
Total of the month	18	22	22	45	40	3	150

Table XX. Results on Clinical Cases

1 May 1950 to 31 May	Honjo	Komagome	Toshima	Toyotama	Ebara	Denken	Total
NEP*	41	72	38	35	20	1	307
<u>Sh. paradyesen.</u> V	1	0	0	0	0	0	1
<u>Sh. paradyesen.</u> VZ	0	0	0	0	1	0	1
<u>Sh. paradyesen.</u> W	4	10	2	2	7	0	25
<u>Sh. paradyesen.</u> WX	5	7	4	1	1	0	18
<u>Sh. paradyesen.</u> Z	1	0	1	0	0	0	2
<u>Sh. paradyesen.</u> Boyd-103	0	0	0	1	0	0	1
<u>Sh. sonnei</u> I	4	2	2	1	3	0	12
<u>Sh. ambigua</u>	1	0	0	0	1	0	2
<u>S. typhosa</u>	1	0	0	1	0	0	2
<u>S. paratyphi</u> B	0	0	1	0	0	0	1
<u>Paracolobactrum</u> inter-medium	1	0	1	1	0	0	3
Total of the month	59	91	49	42	33	1	275

Table XXI. Results on Clinical Cases

1 June to 24 June, 1950	Honjo	Komagome	Toshima	Toyotama	Ebara	Denken	Total
NEP*	60	9	18	30	20	0	137
<u>Sh. paradyesen.</u> VZ	1	1	0	5	2	0	9
<u>Sh. paradyesen.</u> W	10	4	5	4	5	0	28
<u>Sh. paradyesen.</u> WX	10	3	5	3	0	0	21
<u>Sh. paradyesen.</u> Z	1	0	0	0	2	0	3
<u>Sh. sonnei</u> I	9	0	2	0	0	0	11
<u>Sh. ambigua</u>	1	0	0	0	0	0	1
<u>S. paratyphi</u> A	0	0	1	0	0	0	1
<u>S. enteritidis</u>	5	0	0	0	0	0	5
<u>Paracolobactrum</u> inter-medium	1	0	0	0	0	0	1
Paracolon with <u>Sh. Sachs</u> Q771 factor			1	2	0	0	3
Total of the month	98	17	32	44	29	0	220
Total of the Survey	205	172	133	171	152	18	851

* No enteric pathogens

Table XXII. Enteric Pathogens Recovered

	<u>3 Mch-31 Mch</u>	<u>1 Apr-30 Apr</u>	<u>1 May-31 May</u>	<u>1 Jun-24 Jun</u>	<u>3 Mch-24 Jun</u>
<u>Shigella</u>	32	30	62	73	197
<u>Salmonella</u>	26	7	3	6	42
Paracolon	<u>0</u>	<u>1</u>	<u>3</u>	<u>4</u>	<u>8</u>
Total	58	38	68	83	247

SHIGELLA SPECIES AND SEROTYPES IDENTIFIED IN TOKYO, JAPAN: Numerous studies on the distribution of Shigella have been made in recent years. For the most part these emphasize the predominance of Shigella flexneri (S. paradysenteriae) types, the paucity of S. dysenteriae (Shiga), the fluctuating, but usually considerable incidence of S. sonnei and the rarity of the types of Boyd and Sachs (22-23). Weil (16) presented an excellent summary of results compiled to 1946. Shigella isolated in this laboratory prior to 1949 (28) demonstrated that S. flexneri serotypes II was the most common (approximately 40% of those isolated) followed closely by S. sonnei (which comprised about 35% of the isolates). Barksdale (24) has indicated, without reference to incidence, those species which have been isolated in Japan. They included virtually all of the important types.

During the period from January 1949 to 1950, the 406th Medical General Laboratory located in Tokyo, Honshu, Japan, isolated or was called upon to identify 499 Shigella cultures. Three hundred and fifty-one isolates were obtained from Japanese National personnel, while 148 were received from American Occupationaires. These cultures were not obtained in routine survey or from large outbreaks, but were isolates derived from single specimens or those submitted in small numbers. Cultures for identification or verification were obtained from outlying districts in Japan, Okinawa, Philippines, etc. These represent, however, less than 10% of the cultures herein reported.

The organisms were identified by biochemical and serological means. The specific typing sera were prepared either in this laboratory or obtained from the Army Medical Center, Washington, D.C. The slide agglutination method was employed.

In Table XXIII is presented the frequency of the species isolated in both American and Japanese personnel. Approximately 77% of the isolates were S. flexneri. Shigella sonnei was the next most abundant species making up a total of 16% of the isolates. Other species were found in fewer numbers: S. dysenteriae I and II 3.2%, S. alkalescens 2.6%, S. dispar I and II 0.6%, and S. boydii 0.2%.

Table XXIII. Shigella Species Isolated or Identified in 406th Medical General Laboratory, Tokyo, Japan, January 1949-June 1950

<u>Organism isolated</u>	<u>American</u>	<u>Japanese</u>	<u>Total</u>	<u>Percentage of Total</u>
<u>S. flexneri</u> I - VI	87	296*	383	76.8
<u>S. sonnei</u>	34	46	80	16.0
<u>S. dysenteriae</u> II	12	4	16	3.2
<u>S. alkalescens</u> I	12	1	13	2.6
<u>S. dysenteriae</u> I	1	2	3	0.6
<u>S. dispar</u> I	1	1	2	0.4
<u>S. dispar</u> II	0	1	1	0.2
<u>S. boydii</u> I	<u>1</u>	<u>0</u>	<u>1</u>	<u>0.2</u>
Total	148	351	499	100.0

* Includes one culture of S. rabauleensis

Shigella dysenteriae: No isolations of the Large-Sach's types (S. dysenteriae III - VII) were made. Insofar as we can ascertain these types are absent or at least exceedingly rare in Japan. Numerous paracolon organisms have been identified in this laboratory with major Sach's factors, most usually Q771. At least one outbreak of diarrhea was associated with a paracolon with thermostable Q771 factors. The incidence of true Shiga dysentery is at present very low in Japan. This is evidenced by our isolating only 2 strains of S. dysenteriae I (Shiga). On the other hand, 15 isolates of S. dysenteriae II (S. ambigua) were made. This species comprises 8% of the isolates from Americans but only 0.9% from Japanese.

Shigella alkalescens: Only those organisms identified as S. alkalescens I have been recorded, due to the doubtful classification of S. alkalescens II and III. Thirteen isolations of type I were made. The 3.1% approximates that obtained in other locales (15, 19) where this species has been recorded. Again 8% recoveries from Americans were S. alkalescens while only a single specimen (0.3%) was obtained from Japanese.

Shigella sonnei: Next to S. flexneri, isolates of S. sonnei were the most abundant. Our figures (16.0%) approximates those given for the Middle and Far East in general (23). Whereas 23% of cultures derived from Americans were Sonne's bacillus, 13% of the total Japanese isolates were this species.

Shigella flexneri: As noted previously 383 of 499 isolates were identified as being S. flexneri. This figure (76.8%) approximates the average compiled by Weil (16) in results obtained from throughout the world. A breakdown of S. flexneri (Table XXIV) finds serotypes II (W of Andrewes and Inman) to be the most dominant. Two hundred and sixty-nine were this type. It was the most common type isolated from both American (60%) and Japanese (73%).

Table XXIV. Serotypes of S. flexneri Isolated or Identified in
406th Medical General Laboratory, Tokyo, Japan, January 1949-June 1950

<u>Serotype</u>	<u>American</u>	<u>Japanese</u>	<u>Total</u>	<u>% of Total</u>
I (V / VZ)	12	33	45	11.8
II (W)	52	217	269	70.3
III (Z)	11	38	49	12.7
IV (103)	10	5	15	3.9
V (P 119)	0	0	0	0
VI (88)	2	0	2	0.5
Y	0	2	2	0.5
<u>S. rabsaulensis</u>	<u>0</u>	<u>1</u>	<u>1</u>	<u>0.3</u>
Total	87	296	383	100.0

The overall recoveries, 11.8%, of serotype I was approximated by figures from both Americans and Japanese. Although no figures are presented, the majority of the 45 isolates were of the so-called VZ (I, III) type. The type III organisms accounted for 12.7% of the Flexner strains; again with the ratio of American and Japanese being about equal.

Only 15 identifications of type IV (Boyd 103) were made. Ten of these were isolated from Americans, while but 5 were recovered from more than 3 times as many Japanese. It is our feeling that this type is not common in the native population. A goodly number of the specimens failed to ferment mannite.

Whereas the percentage (3.9%) of isolates of type IV fell within the usual limits, the failure to isolate type V and the scarcity of type VI is noteworthy. The latter type is particularly common, especially in Europe and the U.S. We had previously found it to

be the dominant serotype isolated in France (15). Of the two isolates herein recorded one was Newcastle (acid and gas in glucose) and one Manchester (acid and gas in glucose and mannitol).

While 2 strains of the Y race were identified, no isolates of X were made. It is conceivable that the dominance of the II type (WY) has resulted in the occasional isolation of degraded (loss of specific factor) types of II which are identified as Y.

One isolate of a culture described as S. rabaulensis was made. Further work is being conducted to ascertain if this is a true serotype. The patient from which it was isolated, a 3 year old Japanese male, died before further isolations could be made.

Comment and Conclusions - The high incidence of S. flexneri serotypes from both Japanese and Americans in Tokyo (76.8%) is not inconsistent with figures obtained throughout the world. The preponderance of type II in both Americans and Japanese is noteworthy and one can surmise that this is the main indigenous type at the present time. Of S. flexneri types, only IV (Boyd 103) has a discrepant distribution. Whereas this type represented approximately 12% of the S. flexneri types isolated from Americans, it entails only 2% isolated from Japanese.

Shigella sonnei found essentially even distribution in both Americans and Japanese, and represented a large percentage (16%) of the overall isolates. There were significantly more isolations made of S. dysenteriae II and S. alkalescens I in Americans, than were recovered from Japanese. Each of these two types constituted 8% of the total number isolated from Americans in contrast to rates in Japanese of 1% and 0.3% respectively.

The low incidence of S. dysenteriae I, S. dispar, and S. boydii seems to be in keeping with results found elsewhere.

THE ISOLATION OF THREE SHIGELLA PARADYSENTERIAE SEROTYPES FROM AN INDIVIDUAL: On about December 6, 1949 a patient was admitted to the 155th Station Hospital, Yokohama, with moderately severe dysentery. The stools were frequent, grossly bloody, containing much mucous. Fecal material was streaked directly to S.S. agar and EMB agar plates. On the appearance of non-lactose fermenting colonies the plates were submitted to this laboratory.

Four colonies were picked to Kligler's from the S.S. agar plate (S-1, S-2, S-3, and S-4) and two from the EMB plate (E-1 and E-2). S-1, S-2, and S-4 gave typical Shigella reactions, while S-3 and E-2 demonstrated gas formation along with the typically alkaline slope. Culture E-1 was a strain of E. coli. The sucrose-mannitol agar inoculations indicated that S-1 fermented neither of these carbohydrates, S-2, and S-4 fermented mannitol with the formation of acid only, while S-3 and E-1 produced gas on the fermentation of mannitol.

Preliminary slide agglutination tests found culture S-1 to be Shigella paradysenteriae type IV (Boyd 103), S-2 and S-4 S. paradysenteriae type I, III (VZ) and S-3 and E-1 S. paradysenteriae type VI (Boyd 88). The cultures were purified by restreaking on tryptose agar and definitive biochemical and serological studies carried out. It was verified that S-3 and E-1 fermented both dextrose and mannitol with the evolution of gas; thus, Manchester strains. The type IV culture failed to ferment mannitol, a characteristic of most Japanese strains of this type. The immunological characteristics remained as previously indicated.

It is well known that large outbreaks of bacillary dysentery are often not caused by a single type. On previous occasions we have isolated two serotypes from an individual, but we are not aware of three types previously being isolated from a single case. The man from whom the organisms were isolated had had an attack of dysentery some ten weeks previously but had not been hospitalized. It is possible that he was harboring one or more of the types during the interim and has been the victim of a reinfection at a later date. It is obvious that multiple infections could cause havoc with any definitive epidemiological studies. Also, the value of selecting several colonies for study is evident. It is perhaps worthy of note that three subsequent stool

specimens spaced four days apart following sulfonamide therapy found only the type I, III organisms isolated, even though twenty or more colonies were selected for study.

THE GROUP PHASE OF SHIGELLA PARADYSENTERIAE TYPE W: ITS ISOLATION FROM MAN:

Phase variation within the species of Shigella paradysenteriae probably has been described by Boyd (7) as a loss-variation. It was he who pointed out that the loss of the specific type factor of serotype 103 resulted in a variant which had a broad group antigenic mosaic. It was his feeling that the loss of the specific factor allowed the agglutinating potentiality of the group fractions to make themselves manifest. Boyd further showed that in reality the group factors were present in the A (specific) phase and could be demonstrated by preparing an immune serum against the B phase. When this was done antibodies reacting against many group components, not previously detected or which reacted weakly were shown. The 103 A phase threw off group as well as specific variants; the B phase bred true, yielding only group phase organisms.

Weil, Farsetta and Knaub (8), were able to confirm Boyd's work with his type 103. Their attention to the B phase was brought about by the ability of these cultures to be agglutinated by many supposedly specific Shigella typing sera, a situation which also prompted the present investigation. Veazie (9) has likewise described the production of a group phase organism in 103 which was indistinguishable from the X race.

Takita (10) has described variants of V, W, and Z which he interpreted as proceeding in the opposite direction, i.e., the gain of specific factor and a loss of group factor. The direction of variation apparently depends upon the point of view. Boyd (7) fails to agree with Takita's argument concerning a factor gained, pointing out that the loss variation hypothesis as described by him can be applied to Takita's experimental evidence.

The phase variation described by these authors occurred only in old stock cultures where continued growth on artificial media and continued replating apparently stimulated the formation of the group variant. The present communication concerns the isolation of S. paradysenteriae type W in phase B from a case of human dysentery. We are not aware of the previous isolations of Shigella phase B organisms from the body (unless one assumes X and Y races to be group B organisms) except for S. sonnei, in which phase I corresponds to the A phase and phase II to B. There seems little doubt that the phasic variation described for S. sonnei is similar to the phasic A to B variation of the S. paradysenteriae types. The demonstration of the close antigenic similarity between the group phase of serotypes W and that of 103 will be made evident.

Experimental - In September 1949, an enlisted man was admitted to a neighboring station hospital suffering from dysentery. On the 12th of September the first fecal specimen was submitted to this laboratory and Shigella paradysenteriae W was recovered therefrom. Over a period of 6 weeks a dozen specimens were submitted at irregular intervals. From the first five specimens the pathogens isolated were typically W strains, agglutinating only in this type-specific antiserum by the slide method. The isolates from the sixth to the eleventh specimens did not correspond to W, but rather reacted in many supposedly specific antisera, suggesting a broad mosaic of antigens. The twelfth specimen was negative for enteric pathogens.

The reactivity of the B phase was reminiscent of the 103 B organisms first described by Boyd (7) and corroborated by Weil et al (8). In Table XXV we have compared the slide agglutinability of the W A, W B and 103 A, 103 B in supposedly specific typing sera. The activity of the B phase was identical, there having occurred strong clumping in V, W, Z, 103, and 88, while the A phases reacted only in their specific antisera. The typing sera so employed has been successively utilized in typing many hundreds of cultures and was not realized to contain group components until the isolation of the group phase.

The 103 strain used in the study was isolated in this laboratory. It fails to ferment mannitol; such a variant has previously been described by Barnes (11) and Nelson (12). On being carried in stock and replated it dissociated into the classical 103 A - 103 B phases. In Table XXVI are listed the biochemical characteristics of the WA, WB, and the W A, W B and the 103 A and 103 B strains. W A and W B were indistinguishable, one from another, as were 103 A and 103 B. Each serotype was readily distinguished from the other. Type 103 fermented maltose, rhamnose, sorbitol, and dextrin and was indol positive. Type W failed to ferment these carbohydrates and was indol negative. In addition type W

fermented raffinose and mannitol; 103 did not do so. The fermentation of glucose, trehalose, and arabinose was accomplished by both types although arabinose was attacked slowly by type W. Other biochemical activities were characteristic of the genus Shigella.

Cross-agglutination tests using W A and W B as antigens against unabsorbed S. paradysenteriae antisera demonstrated to a slight degree the difference in agglutinability of the two phases (Table XXVII). For the most part phase B reacted to a higher titer than did the A phase.

Table XXV. Slide Agglutination of A and B Phase of S. paradysenteriae types W and 103 in Supposedly Specific Serum

<u>S. paradysenteriae</u> phase agglutination	Specific Antiserum					
	V	W	Z	103	P119	88
W A	-	4	-	-	-	-
W B	4	4	4	4	-	4
103 A	-	-	-	4	-	-
103 B	4	4	4	4	-	4

- = no agglutination

4 = complete agglutination

Table XXVI. Biochemical Reactions of Shigella paradysenteriae W (ES-51, ES-72) and Shigella paradysenteriae 103 (ES 129 A and B)

Species	Strain Number	Glucose	Trehalose	Maltose	Sucrose	Lactose	Raffinose	Arabinose	Xylose	Rhamnose	Mannitol	Dulcitol	Inositol	Sorbitol	Dextrin	Glycerin	Gas	Citrate	Indol	H ₂ S	Urease	Motility
W A	ES-51	1*	1	-	-	-	5	7	-	-	1	-	-	-	-	-	-	-	-	-	-	-
W B	ES-72	1	1	-	-	-	6	5	-	-	1	-	-	-	-	-	-	-	-	-	-	-
103 A	ES-129 A	1	1	1	-	-	1	-	4	-	-	-	3	2	-	-	-	-	+	-	-	-
103 B	ES-129 B	1	1	1	-	-	1	-	3	-	-	-	4	2	-	-	-	-	+	-	-	-

* Days required to produce acidity

Table XXVII. Cross-agglutination results using unabsorbed S. paradysenteriae antisera and W A and W B as antigens

Antiserum Antigen										
	V	Z	W A	W B	103 A	103 B	P119	88	X	Y
W A	320*	80	5120	1280	1280	320	40	160	320	1280
W B	640	1280	640	10,240	5120	5120	80	1280	5120	2560

* Reciprocal of highest dilution showing macroscopic tube agglutination after 18 hours incubation at 37°C. The same is utilized in all agglutination and agglutinin-absorption tables.

Antisera prepared against W A, W B and 103 B were tested against the Flexner serotypes (Table XXVIII). It is worthy of note that the activity against all was marked in the W A antiserum. Type Pl19 and 88 were the weakest reactors. The ability of the A phase to stimulate antibody production to high titer for factors which agglutinated in relatively low titer has previously been pointed out by Boyd for 103 A. The antisera prepared against the two B phases gave, within narrow limits, identical reactions. From the cross-agglutination results examined thus far we were struck by the similarity of 103 B and W B. Boyd (7) previously stressed the similar but not identical nature of the B phases of 103 and Pl19.

Table XXVIII. Cross-agglutination and Absorption Results of *S. paratyphenteriae* Serotypes in Antisera Prepared Against W B, W A and 103 B.

Serum	Absorbed with	Titer with Culture									
		V	Z	W A	W B	103 A	103 B	Pl19	88	X	Y
W B	-	5120	5120	5120	20,480	1280	20,480	1280	160	5120	10,240
	W A	2560	2560	-*	5,120	1280	5,120	640	160	2560	2,560
	103 B	-	-	-	-	-	-	-	-	-	-
	X	320	20	-	1,280	80	1,280	-	40	-	320
	Y	160	320	-	2,560	640	2,560	80	40	80	-
103 B	-	1280	640	320	5,120	320	10,240	320	40	640	1,280
	103 A	320	320	20	1,280	-	2,560	40	-	160	320
	W B	-	-	-	-	-	320	-	-	-	-
	X	40	-	40	160	20	160	-	-	-	40
	Y	320	160	-	2,560	20	2,560	40	-	80	-
	103 A/X/Y	-	-	-	80	-	160	-	-	-	-
W A	-	10,240	640	20,480	10,240	2560	5,120	80	640	1280	5,120
	W A	160	20	-	1,280	-	640	-	-	40	160
	W B	640	40	2,560	-	-	80	-	20	-	-
	103 B	320	40	1,280	80	-	-	-	20	-	-
	103 B/V	-	-	2,560	-	-	-	-	-	-	-

* - less than 1:20

Absorption experiments (Table XXVIII) were carried out on the three above-mentioned antisera. Absorption of anti-W B serum by W A removed all activity for the absorbing organism while leaving the other titers virtually undisturbed. It is probable that W A is capable of removing some of the group component antibodies without effecting a noticeable change in titer. Absorption of W B antiserum by 103 B removed completely the homologous and heterologous activity. Because of the tendency to consider X and Y degraded forms containing only group components (7, 13) these types were used to absorb the W B antiserum. By and large, drastic reductions of all titers occurred. With X there was a complete obliteration of activity for W A, Pl19 and X. With Y, reaction against W A and Y were nullified, but the reduction of other titers was in the main less than that accomplished by X.

Absorption of 103 B antiserum by 103 A brought about many reductions in titer, removing the minor 88 factor and components reacting against the absorbing strain. There seems no doubt that this A phase also contains much of the B phase. Whereas the absorption of W B antiserum by 103 B removed all agglutinins, reciprocal absorption showed a factor remaining which reacted against only 103 B. It is apparent that phase B of 103 contained a component or components in addition to those found in W B. That the X type was very similar to, but not identical with the B phase of 103 B was demonstrated by absorption of the antiserum of the latter with X. The reductions in titers were marked and the residuals remaining were of low titer, but all the reactions were strong. There was a complete loss of agglutinins, for Z, Pl19, 88, and X. The absorption with Y removed many components, but not in the overall fashion accomplished by A. Only W A, 88 and Y

were removed completely. These patterns are quite similar to those accomplished with W B antiserum. A triple absorption using 103 A, X and Y removed all agglutinins except for components reacting against W B (1:80) and 103 B (1:160). It has been pointed out previously that W B removes all agglutinins from 103 B antiserum except a component active against 103 B. The triple absorption accomplished above must, then, leave two factors, one common to W B and 103 B, and one possessed only by the latter.

The absorption of W A antiserum brought to light several points. Neither W B nor 103 B was capable of removing all of the heterologous agglutinins although reduction of titers was marked. A double absorption utilizing 103 B and V produced a serum reacting only with W A.

Of considerable interest is the result obtained by absorbing anti-W A serum with the homologous organism. Whereas the specific reaction (and undoubtedly some group components) were removed for the A phase a considerable amount of group component, especially that reacting against the B phase, was left. This apparently demonstrates the inaccessibility of certain antigenic factors in the A phase, both as regards agglutinability and absorption.

The constancy of the two W phases was checked by absorbing the anti-W A serum (which was prepared by using our strain ES-51 as the immunizing antigen) with our culture ES-17, the first organism isolated from the patient. Complete removal of all agglutinins resulted. The phase B antiserum prepared with ES-72 was absorbed with ES-107, the last phase B organism isolated. Again all antibody was removed. This is an indication that the phase A organisms were antigenically stable, as were the phase B organisms. It should be mentioned also that numerous colonies were picked from all of the plates of primary isolation in conformity with our policy (6). No plates showing a mixture of phases were encountered.

Discussion - It is obvious from the results herein reported that the serological relationship between the B phases of W and 103 is extremely close. In fact, except for an additional factor found in 103 B, they are identical. Biochemically, however, these organisms are readily distinguished one from another.

As far as we are aware, except for phase II of S. sonnei, the group phase of Shigella, has not been isolated from man. Both Boyd (7) and Weil et al (8) concur in this. However, Wilson and Miles (14) state, "sometimes the parent culture may remain stable for months or years; at others it throws off B variants on first subculture or even in the body." A survey of the literature has failed to reveal a single previous instance when S. paratyphenteriae phase B was isolated directly from man.

From the experiments conducted it was evident that W B does not contain any specific W factor. This was proven when the absorption of W B antiserum by 103 B removed all heterologous and homologous activity. Of greater concern is the tendency of the A phase to continue to dissociate to phase B, thus making it virtually impossible to prepare a pure A antigen. It is conceivable that antigens prepared with the specific phase will contain varying amounts of B phase. There seems little doubt but that the B phase isolates are identical (based on a unilateral absorption) and that to date differences in A phase have not been demonstrated. Inasmuch as there was approximately a week between the isolation of the last phase A organism and the first W B specimen, one cannot be certain as to when the variant arose. One can be sure, however, in stating that the dissociation did not occur as the result of replating. Nevertheless, the possibility remains that it arose as the result of the primary plating. This seems unlikely, without consideration of a predisposing factor, for if such were the case it could have occurred during the initial five isolations. The repeated isolation of the specific phase previous to 3 October, and the continued isolation of the group phase after this date can scarcely be dismissed as coincidence. As to why the variant arose one can only hazard a guess; possibly as a result of sulfonamide therapy, or the result of a prolonged infection and the possible stimulus of an immune state. Both of these seem unlikely inasmuch as sulfonamide therapy is common, and long standing cases of chronic bacillary dysentery are well known. A continuous study of fecal antibodies in this case never demonstrated coproantibodies in any detectable concentration, thus it may be assumed that they were not a factor in the shift of phases.

One is struck by the possibility that the B phase represents a step toward complete roughness in a S to R variation. We have been unable to detect colonial differences in W A colonies and W B colonies, although it is to be remembered that phase II of S. sonnei has a large irregular colony. The saline stability of the B variants is unimpaired and there are no other R characteristics found in the B phase, yet the loss variation of the A to B shift is reminiscent of the complete S to R variation. The latter represents a loss of specific and even group components (7).

If the hypothesis be postulated that B variants arose as a result of the stimulus of an increasing immunity of the host, it seems remarkable that so few have been isolated. Nevertheless, the majority of Shigella organisms isolated and typed are undoubtedly derived from acute cases. In civilian life it is difficult to follow the course of infection with continuous laboratory studies. In military installations, however, the patient is hospitalized until definitely cured. This allows for continuous study in prolonged cases. We are at present following a case in which S. paradysoenteriae type VZ has been isolated repeatedly. After several weeks of positive cultures of the VZ type, there seems to be a broadening of the group mosaic, which has not yet evolved to the point of true B phase. Also, a culture obtained from the Philippines and identified there as type V has within 2-3 platings reverted to a phase B. We have also isolated a paracolon organism which reacts as a typical phase B S. paradysoenteriae organism in its serological reactions. Without biochemical studies it would have been identified as a group phase member of S. paradysoenteriae.

Conclusions -

1. Isolation of the group phase of Shigella paradysoenteriae W directly from man was made, in 6 instances, following the previous recovery of the specific phase.
2. A comparison of the group phase of S. paradysoenteriae 103 and S. paradysoenteriae W, showed them to be serologically almost identical. Biochemically, however, our representatives of the two serotypes were easily distinguishable, one from the other.
3. It was demonstrated that the specific phase of serotype W contained the group components, but these were masked by the specific substance. This was best illustrated by the inability of the homologous organism to absorb many group components from anti W serum.

NON-MOTILE PARACOLON WITH IDENTICAL SACHS' TYPE Q771 FACTOR: In 1948 report was made from this laboratory (28) of several instances wherein a paracolon type organism with antigenic factors identical with Q771 were the only suspicious organisms recovered from several cases of acute diarrhea. Interest was focused sharply by an outbreak in a group from which the organism in question was obtained from 21 of 26 stool specimens. Seventeen of these were examined microscopically. Sixteen contained mucus, while leukocytes were observed in 15 and red blood cells in 11.

Since then organisms periodically have been isolated, but our attention was brought to the problem when in June, July, and August 1950, sixteen different isolations of the bacteria were made. In all instances in which the organism was recovered there were no recoveries of well-established enteric pathogens, nor have any intestinal parasites been noted. In almost every instance the fecal material contained inflammatory exudate which is associated with bacillary dysentery.

The paracolons, containing Q771 factors, which are isolated in Japan are different from those described by Wheeler and Stuart (27) in that they are non-motile and many have lost, in culture, the ability to produce gas. The paracolon isolates and representative strains of Q771 fermented dextrose, trehalose, arabinose, and sorbitol. The Sachs' type Q771 failed to ferment lactose, xylose, rhamnose, and mannitol, while the paracolon cultures were able to do so. None of the above mentioned fermented sucrose, raffinose, dulcitol, adonitol, or salicin. They were all Voges-Proskauer, urease, citrate and gelatin negative and were non-motile. Strains of Q771 were indol negative while the paracolons formed this substance (Table XXIX). Many of the latter which showed delayed lactose fermentation tended to yield rapid lactose fermenting variants. It was likewise noted that the ability of fresh isolates to form gas was often lost in culture.

Table XXIX. Biochemical Characteristics of Sachs' type Q771 and Paracolon With Identical Antigen Factors

	Dextrose Trehalose Arabinose Sorbitol	Indol Mannitol Rhamnose ^a Xylose Lactose ^a	Raffinose Salicin Adonitol Dulcitol Sucrose	Motility Gelatin Citrate Urease V-P
Sachs' type Q771	/	-	-	-
Paracolon Q771	/b	/b	-	-

^a fermentation usually delayed from 48-72 hours.

^b fresh isolates and the majority of those carried in stock produce a small amount of gas from the carbohydrates

A serological comparison was carried out utilizing Q771 and 5 of the paracolons. Direct agglutination employing the 6 antisera and antigens left little to choose as regards homologous and heterologous reactions. Reciprocal absorptions of each serum by each of the antigens indicated that all 6 were serologically identical. Included in this study was a rapid lactose fermenting variant and its mother culture, one which fermented lactose slowly.

It is our opinion that the paracolon under discussion is a pathogen of man and of considerable importance in this area. The continual isolation of the organism from morbid material in the absence of other recognized pathogens lends credence to this. Likewise, the presence of inflammatory exudate is added proof of its infectious nature. Also, one would expect that the action on the host of the liberated endotoxin from the paracolon would be identical to that of Q771, inasmuch as the somatic antigens are indistinguishable. The classification of the organism poses a problem. Other than the ability to ferment lactose (sometimes rapidly) and the formation of a small amount of gas (not unique in the genus Shigella) other biochemical characteristics are those of Shigella.

DEVELOPMENT OF LACTOSE-SUCROSE BOOSTER BROTH: Routine enteric bacteriology, in this Department, includes the inoculation of fecal material to both SS (Salmonella and Shigella) and MacConkey Agar plates. Following 18 to 24 hours of incubation suspicious colonies (usually 5 from each plate) are picked and transferred to a booster broth; KIA slants, citrate agar and motility medium are inoculated from this booster vehicle. Only those colonies which appear colorless (non-lactose fermenting) on the initial plates are picked. However, some strains of Aerobacter and most Paracolon and Proteus appear colorless as well as Salmonella and Shigella. During the recent survey of Japanese Hospitals the number of specimens examined were sufficient to demonstrate that picking as many as ten colonies from each specimen (5 from SS and 5 from MacConkey's) thence to booster broth, KIA slants, citrate agar and motility medium involved large amounts of media and time. The question arose as to why it would not be helpful to introduce some method of screening the initial plating and the selective studies. The booster broth previously used was not capable of this as it was intended only to increase the number of organisms in a short period of time. The ingredients of the "old" booster broth are as follows:

Tryptose	20.0 gm.
Dibasic phosphate	2.5 gm.
Sodium chloride	5.0 gm.
Dextrose	2.0 gm.
Water	1000 cc.

The "New" booster broth contains:

Tryptose	20.0 gm.
Dibasic phosphate	1.0 gm.
Sodium chloride	5.0 gm.
Lactose	10.0 gm.
Sucrose	10.0 gm.
Brom Cresol Purple (1.6%) ...	0.5 cc.
Water	1000 cc.

Employing the Lactose-Sucrose Booster Broth we are able to eliminate lactose fermenters (usually Aerobacter) and sucrose fermenters (Paracolon and Proteus) before the various other selective media are inoculated. Tables XXX and XXXI demonstrate the efficacy of this medium in "screening" non-lactose and sucrose fermenters.

Table XXX. Four Hours of Incubation

<u>Indicator</u>	<u>Total Colonies Picked</u>	<u>Acid Reaction in Booster</u>	<u>% Elimination</u>
Brom Cresol Purple	469	99	21
Phenol Red	461	112	24
BCP and PR	930	211	23

Table XXXI. Fifteen Hours (Overnight) Incubation

<u>Indicator</u>	<u>Total Colonies Picked</u>	<u>Acid Reaction in Booster</u>	<u>% Elimination</u>
Brom Cresol Purple	724	394	50
Phenol Red	827	357	43
BCP and PR	1621	751	46

Four hours was not sufficient time but still it was useful. After overnight incubation it was possible to eliminate 46% of the colonies which we picked as suspicious pathogens. In this group was included late lactose fermenting coliform organisms and the sucrose fermenting Proteus and Paracolon. Indol reactions were not impaired. It is felt that in any system employing a booster broth (such as employed at the 406th MGL) the Lactose-Sucrose Booster gives added information and eliminates a considerable proportion of the cultures which may have been considered suspicious. No information is available to ascertain whether or not these cultures would have been eliminated by Kligler's with sucrose added. Ninety strains of Salmonella and 62 strains of Shigella were used to check the reaction of this booster and none except Shigella ceylonensis produced an acid reaction.

HOLDING AND TRANSPORT MEDIUM FOR THE ISOLATION OF SHIGELLA: Although several enrichment and holding media have been devised which adequately serve in the isolation of Salmonella, none have proved outstanding in the recovery of Shigella. In our experience no medium has been satisfactory even in the transport of fecal material to the laboratory, much less to serve as a worthwhile holding or enrichment medium.

Bangxang and Eliot (29) compared several of the older holding media with desoxycholate citrate combinations. They found that 1% citrate and 0.5% desoxycholate in a buffered saline solution was superior to 30% glycerol-saline, 3% normal sodium hydroxide solution and ox bile (10%) in preserving the viability of various species of Shigella. They likewise had success in locating healthy carriers which had not been previously detected with glycerine preservations. It was indicated also that mandelic acid solutions might prove useful in this regard.

Brodie (30) has studied extensively a modified Leifson media for the isolation and enrichment of dysentery bacilli. From a total of 93 positive specimens obtained by the combined efforts of direct plating on his rosolic acid citrate agar and by enrichment in a modified Leifson fluid medium citrate neutral red (CNR broth) 68 (73.1%) were obtained by direct plating while only 47 (50.5%) were recovered by enrichment methods. Of the latter, however, 25 were obtainable only after enrichment, being undetected by direct plating. Substituting rosolic acid for neutral red in the enrichment fluid appeared to produce a more efficient medium, citrate rosolic acid (CPA broth). In a small series of 15 positive dysentery stools, the CRA enrichment media yielded 12 positive isolates. This was greater than the 9 obtained by direct plating or the 7 recovered from the CNR enrichment. The CRA broth recovered 4 not obtained by other methods whereas 3 were missed by this means.

It has been recently reported by Brodie (31) that his citrate rosolic acid broth was more effective when used in a shallow layer (2 mm) than in the deep layer.

The medium used by the majority of laboratories (32) for transporting and holding fecal specimens is the buffered glycerol-saline medium. This method has been recommended by Galton (33) and Ewing and Edwards (34). It has been our experience that this medium is only moderately satisfactory for the isolation of Shigella when heavily seeded with fecal material and is of little value in preserving rectal swab specimens unless followed by enrichment for the detection of Salmonella.

In view of the repeated observations that SS agar (Bacto) is an excellent medium for the primary isolation of Shigella from fecal material, it seemed worthwhile to test SS agar as a possible holding and transport medium. At the same time it was deemed advisable to test the efficiency of combined components of the SS formula and to compare also buffered glycerol-saline. The CNR broth of Brodie with modification was employed. Also, it was decided to employ one of the standard Salmonella enrichment media in the test. Selenite broth was the medium selected. The purposes of the experiment was primarily to ascertain that medium which was the most suitable for holding and transporting suspected fecal specimens to the laboratory for the purpose of isolating Shigella organisms. Enrichment was not considered.

Media Employed - Seven media were employed in this comparative study. The first group of specimens utilized six of these, the CRA broth of Brodie not being used. This was occasioned by the fact that we were unaware of the medium and the latest shallow layer technique until the project was underway. The CRA broth was then compared with the direct SS agar plate and the SS agar slant, the latter holding medium having shown the greatest promise during the culturing of the first 100 specimens.

1. SS agar (Bacto) plates. These were made fresh daily.
2. SS agar (Bacto) slant. Approximately 12 ml. of freshly prepared SS agar was placed in sterile 22 ml. screw cap vials and slanted to produce the maximum surface.
3. CNR broth. This medium was the desoxycholate citrate medium of Leifson devoid of agar. It differed from Brodie's CNR broth in the substitution of pork infusion and proteose #3 (Difco) for the serum peptic digest Lemco broth and sodium desoxycholate for sodium taurocholate. Two ml. was dispensed in 22 ml. screw cap vials.
4. CRA broth. This was identical to the CNR broth substituting for the neutral red, in amounts of 0.5 ml. of a 1% solution in absolute alcohol for each 100 ml. of broth.
5. SS broth. The Standard SS agar (Bacto) formula minus the agar. This was dispensed in the same manner as the CNR broth.
6. Buffered glycerol-saline preservative made up according to the formula given by Galton (33). Dispensed in 12 ml. quantities in 22 ml. screw cap vials.
7. Selenite broth (Difco) enrichment medium as described by Leifson was dispensed in the same manner as the glycerol-saline broth.

Source of Material and Method - One hundred and sixty-four fecal specimens were obtained from Japanese patients hospitalized in the Tokyo area. The clinical diagnosis in each case was dysentery.

Each medium was inoculated with a cotton swab which was heavily impregnated with the specimen in question. The SS agar plate was streaked in such a manner as to insure isolated colonies. No attempt was made to achieve isolation on the SS slant, but rather the material was smeared heavily on the surface. Each of the liquid media received a generous portion of the fecal material. All of the media were inoculated at the hospital and returned to this laboratory within a few hours.

The SS agar plates were incubated immediately on return to the laboratory at 37°C. until the following morning. All other media were held overnight at room temperature and then incubated 16-24 hours at 37°C.

Transfer was made from test media to fresh MacConkey agar plates. in the case of the SS slant the total growth was mixed and then a part of the mixture streaked for isolation. After proper incubation the MacConkey plates were examined for non-lactose fermenting colonies. Four or five colonies from each SS and MacConkey agar plate were inoculated into a tryptose-phosphate booster broth containing 1% sucrose and lactose with brom cresol purple as an indicator. After four hours incubation those "booster" broths not showing definite acidity were inoculated to a battery of media. Those cultures which were definitely acid were discarded. The biochemical battery consisted of Kligler's iron agar / 1% sucrose, Simmon's citrate agar and motility agar. After inoculation, incubation was carried out at 37°C. for 16-24 hours. The remaining alkaline "booster" broths were reincubated similarly. At the end of the period of incubation the booster broth was utilized for the testing for indol with Kovac's reagent. From these biochemical reactions suspicious Shigella and Salmonella isolates were kept and the remainder discarded. In this regard it is well to mention that no Salmonella were isolated from the first 100 specimens although the yield of P. intermedius was fairly high. The latter have not been tabulated in this article. Those which showed Shigella reactions were checked in Shigella polyvalent serum and if positive, subsequently identified as to species and type.

Experimental Results - A total of 100 fecal specimens from as many patients were cultured over a 3 week period in June-July 1950. Forty-six Shigella were recovered by all media, i.e. SS agar plates, SS slant, SS broth, CNR broth, Glycerol-Saline broth and Selenite broth, the second from CNR only, and the third from CNR broth and the glycerol broth. Thus 93.5% of recoveries were made by direct plating. Only the SS slant yielded more than 50% positive isolates where 36 (78.2%) were detected. The buffered glycerol-saline yielded 23 (50%), SS broth 21 (45.7%), and CNR 19 (41.3%). The Selenite broth enrichment, as might be expected from previous experience was the most deficient as only 11 (23.9%) Shigella were recovered from it. Definitive typing of the Shigella isolated showed: 9 to be S. flexneri I, 27 S. flexneri II, 4 S. flexneri IV, 7 S. flexneri X and 5 S. sonnei.

Table XXXII. Recovery of Shigella from 100 Fecal Specimens by Direct Plating and 7 Holding Media

<u>Medium</u>	<u># Isolates</u>	<u>% Total Isolates</u>
All media	45	100
SS agar plate	43	93.5
SS agar slant	36	78.2
Buffered glycerol saline	23	50.0
SS broth	21	45.7
CNR broth	19	41.3
Selenite-F	11	23.9

The holding qualities of the SS slant were tested further by seeding fresh fecal specimens with Shigella, streaking the slant and holding at room temperature for one week. Six slants were streaked with a specimen seeded with S. paradyse-teriae II and six with feces seeded with S. sonnei phase I. The slants were incubated at 37°C. and each day a portion of the growth was streaked to MacConkey's plates and suspicious colonies handled as mentioned previously. It was found that the media maintains Shigella in three instances for one week at least. On the sixth day, for example, 11 of the 12 slants were positive, although on the fifth day only 7 of the 12 were positive. The latter figures also prevailed for the seventh day. The inconsistent results can be best explained by the fact that growth was not mixed before re-streaking, but rather a random loop of material was taken for the inoculum.

The effect of age of the SS slant was also investigated. The bottled slants were prepared, one set kept at room temperature and another in the ice box at 4°C. At the end of 3 days 6 slants were inoculated with fecal material artificially seeded with S. paradyse-teriae II and 6 with material containing S. sonnei. After overnight incubation at 37°C. it was possible to reisolate the type II from 5 of the 6 slants and S. sonnei from all six.

A set of slants which had been held 7 days at room temperature and another set held in the ice box for the same time were tested in the above manner. All slants held at room temperature did not allow recovery of S. paradyse-teriae II, but S. sonnei was recovered from 3 of 6 slants. The set which had been held in the ice box before inoculation yielded type II in all but one instance and S. sonnei from all 6 slants.

In further evaluation of Brodie's CRA broth, SS agar plates and bottled SS agar slants a total of 64 suspect stool specimens were next tested. Of these 41 were positive for Shigella by direct plating, while 29 were detected on replating to MacConkey's from the CRA broth; 30 were recovered from the SS slant. There were no positives detected by CRA broth or the SS slant that were not accounted for by direct plating. From the CRA broth 3 Shigella were recovered which were not revealed by the SS slant, while 4 were obtained from the latter which were not recovered from the former. (Table XXXIII)

Table XXXIII. Comparison of S.S. Agar Slant and Citrate Rosolic Acid Broth as Compared to S.S. Agar Plate in Recovering Shigella

Medium	Specimens	# <u>Shigella</u> Recovered	# <u>Salmonella</u> Recovered	% <u>Shigella</u> Recovered
S.S. agar plate	67	41	3	100.0
S.S. agar slant	67	30	1	73.2
C.R.A. broth	67	29	2	70.7

On definitive typing 31 were S. flexneri II (W), 5 S. flexneri I (V), 3 S. flexneri IV (Boyd 103), and 2 S. sonnei.

Salmonella were recovered from 3 specimens. These consisted of 2 S. paratyphi A and 1 S. typhosa isolated by direct plating, whereas 1 S. paratyphi A and the typhoid culture were recovered with the CRA broth. The SS slant yielded only one of the two S. paratyphi A isolates.

Discussion - As is to be expected, from the results compiled in this study it is apparent that there is no substitute for direct plating for the isolation of Shigella. Nevertheless, greater success has been attained by use of SS agar slants and CRA broth, than by the frequently used buffered glycerol-saline.

The success of the SS agar slant in recovering 75% of the Shigella from proven positive specimens, is apparently a reflection of the success of the medium when used in the plate. The solid state of the medium serves, it would seem, some worthwhile purpose, as the same formula minus only the agar (SS broth) was less successful. There was, of course, massive overgrowth by other fecal organisms in many instances. The percentage

of recoveries was approached only by Brodie's CRA broth. As pointed out by Halbert (35) there are many coliform which produce antibiotic substances which act against Shigellae. It is possible that the agar prevents the ready diffusion of such substances, a condition not provided by a liquid medium. The use of SS agar in a sturdy screw cap vial provides a means by which a specimen may be transported easily to the laboratory.

There are differences between the broth that we have called CNR and Brodie's CRA broth formula. Essentially it is the use of a special serum peptic digest and sodium taurocholate in the CRA formula and the use of proteose #3 peptone and sodium desoxycholate in the CNR broth herein reported. Nevertheless, the recovery rate is as determined by Brodie (47 of 93, 50.5%) and the recovery rate reported here for CNR broth (19 of 46, 41.3%) are within limits not too widely divergent. It is also to be noted that the replating medium used in this study was MacConkey's agar; Brodie used citrate rosolic acid agar in his study.

The failure of Selenite broth was not unexpected inasmuch as the medium was devised to aid in the recovery of Salmonella. Yet, one speculated as to the number of laboratories expecting aid in Shigella isolation by use of the formula (32).

The relatively poor results obtained with buffered glycerol-saline solution, was not foreseen. It has been recommended by others as the preferable preservation means (32). Certainly the significant differences obtained between it and direct plating indicate that too much confidence has been placed in the procedure. Our attempts to isolate Shigella from rectal swab cultures which have been preserved in buffered glycerol-saline (33) have resulted in almost total failure. It was found that the plating of swabs so preserved yielded extremely poor results, often showing no growth whatsoever. We believe that this may be due to the transfer of enough of the glycerine solution to the plate to effectively inhibit most growth. This is obviated (for Salmonella at least) by first transferring swabs to a satisfactory enrichment medium.

Summary -

1. A comparative study has been made using various media to determine which affords best recovery after delay in definitive attempts at isolation. A total of 164 clinical cases were the source of fresh material for the study.
2. In comparison with the 93.5% recovery effected by direct streaking on SS agar plates in 100 cases the recovery elicited by other holding media was as follows:

SS agar (Bacto) slants	78.2%
Glycerol-saline broth	50.0%
SS broth	45.7%
Citrate Neutral Red broth	41.3%
Selenite broth (Bacto)	23.9%

3. In 64 additional cases, Citrate Rosolic Acid broth showed 45% recovery in comparison with direct plating on SS agar.

A METHOD FOR DELAYED CULTURAL IDENTIFICATION OF NEISSERIA GONORRHOEAE: A study has been in progress in this laboratory for the past two years (28) to determine a practical method for the collection and shipment of suspect material for cultural identification of Neisseria gonorrhoeae. From the early stages of this study it appeared that the problem included search for both an improved receptacle and medium. It has been our experience that the usual petri dish is not too suitable for handling by other than bacteriologists or trained technicians, and the fragile nature of the receptacle prevents its adaptability to shipment under the usual conditions found in field military medical installations. After continued close contact with this problem it became apparent that the desirable receptacle should include the following:

1. Sufficient strength and rigidity to allow simplified packing and shipment.
2. Reduced opportunity for contamination.
3. Sufficient surface area to allow isolation of fastidious organisms despite rapid growth of contaminants.

4. Preservation of medium under storage conditions prior to use.

A screw cap container was immediately suggested as meeting several of these criteria. Screw cap test tubes, however, presented too small a surface of medium. A two ounce screw capped prescription bottle was used, each containing 10 ml. of solid medium distributed on the broad rear surface. This provided a layer of agar 3 ml. thick. If bottles of reasonably good manufacture were used suspect gonococcal colonies could easily be detected without visual distortion.

The media employed in the several tests mentioned herein were as follows:

Medium #1 - McCleod's Chocolate Agar (36)

Base: Proteose #3 agar 45 gm.
Agar (Bacto) 5 gm.
Distilled water 1000 ml.

Dissolve the proteose #3 and plain agar in water and dispense in desired quantities. Autoclave at 15 lbs. for 20 minutes. Add 7.5% blood and bring resulting solution to 80°C. and maintain at this temperature until medium is a chocolate brown color.

Medium #2 - Blood Agar

Tryptose blood agar base:

Tryptose (Bacto) 10 gm.
Beef extract 3 gm.
Sodium chloride 5 gm.
Disodium phosphate 5 gm.
Agar Bacto 17 gm.

Dissolve base in 1000 ml. of distilled water and sterilize in autoclave at 15 lbs. for 15 minutes.

To prepare blood agar allow base to cool to 45°C. and add 7.5% human blood.

Medium #3 - 406th Medium (McCleod's Medium Utilizing 7.5% human Blood plus 10% Yeast Extract)

Medium #4 - Carpenter's Medium - II (37)

Component 1.

Base:

Proteose peptone #3 15 gm.
Sodium chloride 5 gm.
Dipotassium phosphate 4 gm.
Monopotassium phosphate ... 1 gm.
Agar (Bacto) 20 gm.
Corn starch 1 gm.
Distilled water 500 ml.

Melt agar and dispense 100 ml. amounts in $\frac{1}{2}$ liter flasks. Autoclave at 15 lbs. for 15 minutes.

Component 2.

Hemoglobin (Bacto) 10 gm.
Distilled water 500 ml.

Agitate for 10 minutes or longer at room temperature and filter through gauze to remove undissolved particles. Dispense in 100 ml. amounts in $\frac{1}{2}$ liter flasks. Autoclave at 15 lbs. for 15 minutes.

To prepare final medium mix components 1 and 2 at 50 to 66 degrees centigrade in equal amounts and add 1% supplement B (Difco).

Material for inoculation was obtained from the cervical discharge of 100 females for each of 3 test surveys. These individuals were all considered clinically to be infected and undergoing treatment at a Japanese hospital. Care was taken in each of the surveys to insure application of material to culture media in an impartial manner by serial alternation with the control plates being the initial medium streaked.

Cultures were incubated in large dessicators at 37°C. with increased CO₂ supplied by candle. The screw cap of each bottle was loosened to permit the pervading of the CO₂ within the bottle. All cultures were examined at 24 hours and typical Neisseria gonorrhoeae colonies picked to unaltered chocolate agar for purity. Cultures negative at 24 hours were reincubated an additional day and re-examined. If microscopically negative a standard oxidase test was carried out. Subcultures from these colonies were also made with care taken to insure their viability by early picking. Pure cultures were characterized as gonococci by the heavy inoculation just beneath the meniscus of dextrose, maltose, and sucrose semisolid media. The carbohydrates were routinely read at 24 hours but by use of the heavy inoculum readings were always possible at the end of three hours. Fermentation of dextrose with no action on maltose or sucrose was utilized to verify the presence of Neisseria gonorrhoeae.

Experimental Results - An incomplete study of 100 cases in 1948 (Table XXXIV) showed the 406th Medium to excell slightly McLeod's Medium in both plates and bottles incubated immediately and held 24 hours at room temperature before incubation at 37°C. in the candle jar. The relatively small loss of possitivity (7% by the delay in incubation) occasioned a comparison in 1949 of the then newly advocated Carpenter's II agar (37) with the 406th Medium. Little difference in recovery for Carpenter's (62%) and 406th (63%) (Table XXXV) for bottles led to a more controlled series of 100 cases in 1950 involving the 2 media.

Table XXXIV. Comparison of Three Media, Plates, and Bottles, Immediate and Delayed Incubation

Overall	Control Plates			1948 100 cases Bottles immediate inc.			Bottles held R ^o 24 hrs.		
	Med. #1 (Mc Leod's)	Med. #2 (Blood Agar)	Med. #3 (None)	Med. #1 (Mc Leod's)	Med. #2 (BAP)	Med. #3 (406th)	Med. #1 (Mc Leod's)	Med. #2 (BAP)	Med. #3 (406th)
Positiv- ity (all media)*									
60%	58(58%)	58(58%)	-	49(84.4%)	46(79%)	51(89%)	35(71%)	29(63%)	44(86%)

* 2 strains recovered from test media not recovered on control plates.

Table XXXV. Recovery of Neisseria gonorrhoeae from 2 Holding Media

Overall	1949 100 cases					
	Pos. plate cultures 406th plates Medium #3		Medium #3 (406th medium) Bottles % Recovery of positive ∇ Cases		Medium #4 (Carpenter's) Bottles % Recovery of positive ∇ Cases	
Positiv- ity (all media)						
81%	81	81%	51	63%	50	62%

Control plates and bottles of both media incubated immediately at 37°C. under reduced O₂ tension resulted in a total of 72 positive Neisseria gonorrhoeae cultures. Bottled media of both delayed 24 hours showed the 406th Medium to be very slightly more efficient than Carpenter's II agar. As can be seen in Table XXXVI, recovery in positive cases was 75% for the 406th Medium as compared with 71% for Carpenter's.

Table XXXVI. Cultural Study of Two Solid Media Reference Facility as
"Holding" Agent and Growth 24 Hours

Plates at 37°C.	1950 100 Cases				
	All cases # Pos.	% Pos.	% Recovery in Pos. Cases	# Growth in 24 hours	% Growth in 24 hours
Total Pos. plates	72	72	-	-	-
Medium #3 (406th plates)	71	71	98	42	59.2
Medium #4 (Carpenter's plates)	71	71	98	40	56.5
Bottles immed. incub. 37°C.					
Total Pos. bottles	72	72	100	-	-
Medium #3 (406th bottles)	68	68	94.5	60	87.8
Medium #4 (Carpenter's btl.)	66	66	91.5	57	86.1
Bottles held at R ⁰ 24 hrs.					
Total R ⁰ Pos. btl.	61	61	85	-	-
Medium #3 (406th bottles)	54	54	75	28	51.6
Medium #4 (Carpenter's btl.)	51	51	71	22	43

Discussion - It has not been the sole purpose of these studies to develop a bacteriologic holding medium superior to the many reported in the literature. The need of the Army Medical General Laboratory, an installation analogous to a State Health Laboratory, is for a medium which will maintain viability for 24 hours before proper incubation, will permit ease of storage and shipment and lend itself to compact enclosure in a vessel such as a dessicator or mayonnaise jar. The 406th agar and/or Carpenter's II agar encased in a screw cap 2 ounce bottle apparently satisfies this need admirably. The screw cap medicine bottle allows for sufficient streaking surface, provides a tight sealing of the bottle during the holding period which permits contaminating flora to provide a reduced O₂ tension and also permits the free egress of CO₂ in the dessicator when the cap is slightly loosened during incubation. The bottle solves the problem of soaked cotton plugs and serves to retain moisture. In this connection impaired visibility due to steaming at times makes the picking of colonies difficult. This has been obviated by removing the film of moisture by the use of sterile absorbent cotton applicators.

The sturdy feature of the bottle container permits large numbers to be shipped in ordinary carboard containers from field installations where meticulous handling is not always possible. The bottled solid agar has several advantages over tubed broth. It prevents dilution of urethral exudate to the point where lightly seeded specimens may fail to grow on transfer. The chore of streaking from broth to plate with attendant possible contamination is eliminated. Moisture is present and evaporation is held to a minimum by the screw cap. This is sufficient to provide the one recommending feature of the liquid medium. However, evidence seems to be accumulating that in spite of many nutrients, loss of viability occurs less often after a sojourn on solid media than in a liquid transport vehicle.

Summary - 1. A 2 ounce prescription type screw capped bottle is a practical and desirable receptacle for gonococcal holding media affording excellent recovery in comparison to that obtained on standard petri dishes.

2. A modified McLeod's medium containing blood and yeast extract allows 75% recovery after 24 hours delay in incubation.

3. Bottled media appears to offer the most practical method for handling referred cultures initially prepared in those field installations which permit only cursory streaking and dispatch through mail or other means of shipment.

A SAFE HYDROGEN GAS GENERATOR FOR THE BREWER ANAEROBIC JAR: With the outbreak of hostilities in Korea it became apparent that the resulting number of contaminated war wounds would greatly tax the existing facilities in this laboratory for anaerobic culture and would necessitate a modification of the gas assembly apparatus required to prepare the Brewer Anaerobic jars. As described by Brewer (3) the anaerobic jar provides the optional use of illuminating gas or hydrogen burned by action on an electrically heated platinum catalyst encased in the cover assembly. When illuminating gas is used a preliminary evacuation is necessary before the gas can be drawn into the jar. In the use of hydrogen generated by the apparatus sketched in Figure 2, the gas is admitted at a pressure of 1 to 2 lbs. per square inch indicated by 10 to 20 cm. of Hg. on the monometer (A) after passing through a wash bottle (C). The combustion is indicated by steaming of the jars resulting from condensation of water formed by oxidation of the gas. At this point a methylene blue indicator tube previously placed in the jar along with the cultures will become colorless representing obligate anaerobiosis. The difficulty inherent in any hydrogen generator is inability to regulate the flow of gas at the desired pressure. Too vigorous operation of the generator may result in explosion. The necessity of charging several Brewer jar simultaneously requires quite active hydrogen generation which in itself enhances probability of explosion. This hazard has been eliminated in this laboratory by incorporating a pressure control tube (B) in the apparatus. This device is designed to allow free escape of excess gas through a column of mercury equal to the height in centimeters of Hg. in the manometer (i.e. 10.0 cm. Hg. in manometer equals 1 lb. of pressure). The pressure within the control tube may be increased or decreased by simply adjusting the length of the glass tube conveying the gas into the safety device (B). Hydrogen gas is used exclusively in this laboratory rather than the more cumbersome illuminating gas method requiring some type of pump for initial evacuation of the jar. H₂ gas is perfused for 30 minutes and burned on the heated catalyst. This arbitrary time limit works as well for 1 as for 5 jars charged simultaneously so long as a pressure of 10 to 20 cm. of Hg. is constantly maintained.

BACTERIOLOGY OF WAR WOUNDS: ANAEROBIC CULTURE ROUTINE: A standard routine procedure for the isolation and identification of organisms of the genus Clostridium has been established in the Bacteriology Department. Particular emphasis is placed on detection of members of the gas gangrene group, including Cl. perfringens, Cl. septicum, Cl. novyi (oedematiens) and Cl. sordelli (bifermentans). Cultural examinations and animal inoculations are carried out as follows.

Isolation - Specimens are received either in the form of portions of tissue or swabs from wounds, or as cultures in thioglycollate medium, litmus milk, or cooked meat broth. In the case of tissues or swabs, they are inoculated into cooked meat broth tubes and incubated aerobically* at 37 C. for 24 hours, then subcultured in serial dilution to two sodium-azide blood agar plates. Such specimens are also initially inoculated into tubes of glycerol broth. The azide-blood agar plates are incubated anaerobically for 48 hours in a Brewer jar charged with hydrogen. Those specimens received in the form of previously inoculated cultures are subcultured to two azide-blood agar plates in serial dilution, as well as to a tube of glycerol broth.

As a presumptive test for the presence of Cl. perfringens, the initially inoculated tube of glycerol broth is tested at 6 and 24 hours of incubation for acrolein aldehyde (4) (see section on "Acrolein Test for Cl. perfringens"). A positive test at either time indicates the presence of Cl. perfringens; a negative reaction does not preclude the possibility of the presence of this organism, but may occur when the initial specimen contains extremely small numbers of bacilli. Acrolein tests are also carried out

* In the event that the presence of Cl. tetani is specifically suspected, the initial incubation of cooked meat broth is carried out anaerobically, although this species has been successfully isolated after initial aerobic incubation.

"HYDROGEN GAS GENERATOR FOR ANAEROBIC JAR"

"Regulator consists of the tube which can be moved up or down through one hole of a two hole stopper fitted tightly in the mouth of a test tube containing mercury."

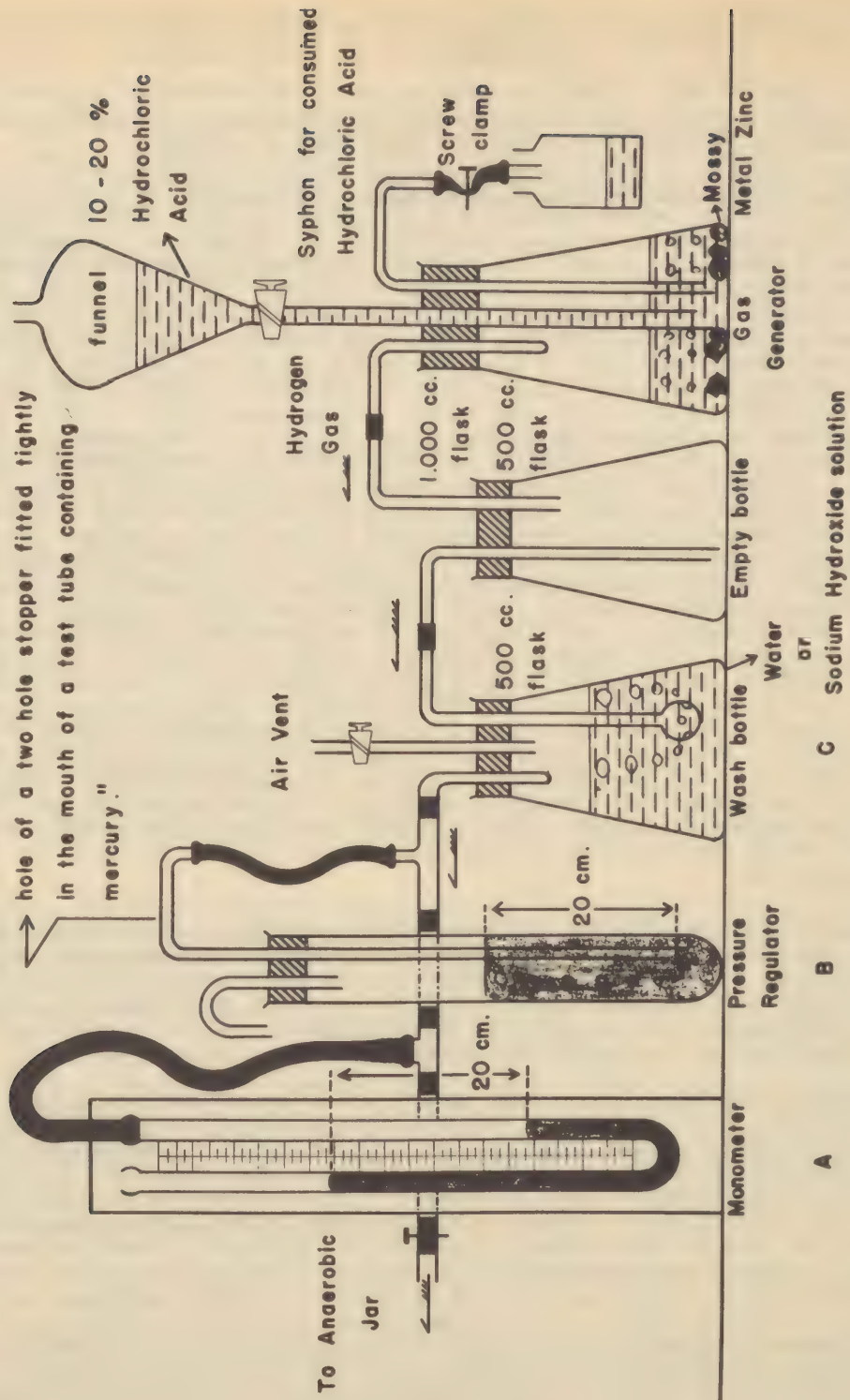


FIGURE 2

on glycerol broth cultures of liver and muscle from guinea pigs dying after inoculation with tentatively identified Cl. perfringens isolates, as a rapid confirmation of the identity of the suspected organism.

The initial azide-blood agar plates, inoculated from cooked meat broth or from the original cultures as received, are examined after 48 hours of anaerobic incubation. Isolated colonies of potential Clostridia are examined by Gram's stain; those showing apparently pure suspension of Gram-positive bacilli are transferred to tubes of thioglycollate broth. A particular effort is made to pick colonies of all morphologic types present. Two colonies of each possible Cl. perfringens strain are fished whenever possible to increase the probability of obtaining a toxigenic strain. Colonies which appear mixed but which include Gram-positive bacilli are subcultured to azide-blood agar plates in order to recover pure cultures of these organisms. Cell morphology and the presence or absence of spores are noted at this time. Gram's stain decolorizing is best done with 95 per cent alcohol; when acetone-alcohol mixtures are used, excessive destaining of Clostridia often occurs.

The thioglycollate broth culture of each single fished colony is incubated for 24 hours. Subcultures are then made to glycerol broth for a confirmatory acrolein test on strains from specimens which had initially given a positive reaction, and to a blood agar plate for aerobic incubation, in order to determine whether the organism under study is an obligate anaerobe or a facultative aerobe. In the event that profuse aerobic growth occurs, a repeat attempt is made to isolate anaerobic bacilli by returning to the thioglycollate broth tube and replating to azide-blood agar plates. If no anaerobic isolates are obtained in the first isolation sequence, the original cooked meat broth is replated and potentially significant colonies are subcultured in the manner outlined in par. 3 et seq. Two or more complete series of isolation attempts on a specimen are made before the search for anaerobic bacilli is discontinued.

If the aerobic plate culture from thioglycollate medium is negative at 24 hours, or in the event that only micro-aerophilic Gram-positive bacilli are present, the culture is deemed suitable for use in determining biochemical reactions. Growth from the thioglycollate pure culture is transferred to individual carbohydrate and other determinative media, and the cultures are characterized as described below.

Biochemical Reactions -

1. Tubes containing the following media are inoculated from the thioglycollate pure culture of the organism under study, and incubated as indicated:

Anaerobic incubation:

- (1) Nitrate broth, for demonstration of reduction.
- (2) Tryptone broth, for demonstration of indol formation.
- (3) Gelatin (0.2 percent) tryptose agar, for demonstration of motility.
- (4) Nagler's (egg yolk) agar plate, for demonstration of lecithinase.

Aerobic incubation:

- (1) Litmus milk, for demonstration of fermentation, coagulation, digestion, and peptonization.
- (2) Tryptose-lactose-iron agar, for demonstration of H₂S production.
- (3) Lactose.
- (4) Glucose.
- (5) Maltose.

(6) Sucrose

(7) Salicin

Sugars are made up in a thioglycollate base. Indicator base. Indicator solution is added after 48 hours incubation to determine whether fermentation has occurred. Test and observation for nitrate reduction, indol formation, gelatin liquefaction, and motility are made at 48 hours.

Test for Animal Pathogenicity - A cooked meat broth tube is inoculated from the pure culture in thioglycollate broth at the time of inoculation of biochemical tests, and incubated anaerobically for 72 hours. One cc. of the supernatant fluid from this tube is injected intramuscularly in the left femoral area of a guinea pig. The site of inoculation is traumatized with a forceps prior to the injection. Guinea pigs are observed for 7 days following inoculation. Animals which remain healthy and show no abnormality at the site of inoculation are discarded at this time.

Guinea pigs dying during the observation period are autopsied and examined for gross pathologic changes representative of gas gangrene infection. Edema, emphysema, and histolysis may be seen. Aerobic and anaerobic cultures of heart blood are made if the volume of work permits. Specimens of liver and muscle, the latter from the site of inoculation, are cultured in glycerol broth which is tested for acrolein, in cases where Cl. perfringens is suspected.

Identification Key - The observations made according to the preceding sections permit characterization of the individual species of Clostridia according to the outline proposed by Spray (5), as modified by Dr. R. P. Elrod.

1. Iron Milk: Active gaseous fermentation, early coagulation (12-48 hours). No digestion of clot, no blackening.

Lead Acetate: Strongly blackened.

Nitrate /, Indol-, Gelatin/, Motility -, Acrolein /, Nagler /.

Glucose /, Lactose /, Sucrose /, Salicin -

Spores ovoid, central-excentric, not swelling rod, infrequently observed and not in fermentable sugars.

Pathogenic to guinea pig.

Clostridium welchii (perfringens)

2. Iron Milk: Inactive gaseous fermentation, late coagulation (4-7 days), no digestion of clot, no blackening.

Lead Acetate: No blackening, no browning.

(1) Motility /

(a) Gelatin /, Nitrate /, Indol -, Nagler - .

Glucose /, Lactose /, Sucrose -, Salicin /.

Spores ovoid, abundant, excentric to subterminal.

Pathogenic for guinea pig.

Clostridium septicum

(b) Gelatin -, Nitrate /, Indol - (some strains /).

Glucose /, Lactose /, Sucrose /, Salicin /.

Spores spherical, sub-terminal to terminal, swelling rod.

Non-pathogenic for guinea pig and rabbit.

(2) Motility -

Nitrate /, Indol -, Gelatin -.

Glucose /, Lactose /, Sucrose /, Salicin /.

Spores rare, ovoid, sub-terminal, swelling rod.

Pathogenicity: variable for guinea pig (pathogenicity is lost on sub-culture).

Clostridium fallax

Lead Acetate: Weakly positive

Nitrate /, Indol -, Gelatin -, Motility /, Nagler -.

Glucose /, Lactose /, Sucrose /, Salicin /.

Spores oval, terminal, swelling rod, micro-aerophilic

Non-pathogenic for guinea pig and rabbit.

Clostridium tertium

3. Iron Milk: inactive gaseous fermentation (long continued). Late, if any, coagulation (10-20-30 days or even 60 days), no digestion, or blackening.

Lead Acetate: strongly blackened.

(1) Lactose -, Nitrate -, Indol -, Gelatin /, Motility /, Nagler /.

Motility /, Nagler /.

Glucose /, Sucrose -, Salicin -.

Spores, ovoid, not abundant, excentric to sub-terminal, swelling rod.

Pathogenic for guinea pig.

Clostridium novyi

(2) Lactose /, Nitrate /, Indol -, Gelatin /, Motility /, Nagler -.

Glucose /, Sucrose /, Salicin -.

Spores ovoid, abundant, excentric to sub-terminal, swelling rod.

Pathogenic for guinea pig and rabbit.

Clostridium chauvei (fessler)

Lead Acetate: No blackening, no browning.

Nitrate -, Indol -, Gelatin /, Motility /, Nagler /.

Spores ovoid, not abundant, terminal, slightly swelling rod.

Clostridium botulinum (C, D, and E)

4. Iron Milk: inactive gaseous fermentation, more or less rapid digestion (with or without previous clotting), strongly blackened early (48 hours) or late (8-9 days).

Lead Acetate: strongly and rapidly blackened (24-48 hours).

- (1) Salicin /, Nitrate -, Indol -, Gelatin /, Motility /, Nagler /.

Glucose /, Sucrose -. Lactose -.

Spores ovoid, not usually abundant, excentric to subterminal swelling rod. (Iron milk softly coagulated (2-5 days), first blackened (5-7 days), clot slowly digested (10-20 days)).

Differentiated by antitoxin.

Clostridium parabolulinum (A and B)

- (2) Salicin -

- (a) Indol -, Nitrate -, Gelatin /, Motility /, Nagler /.

Glucose /, Lactose -, Sucrose -.

Spores ovoid, abundant, excentric to sub-terminal, swelling rod.

(Iron milk not coagulated, translucent then flocculent precipitate, rapidly blackened (24-48 hours), rapidly digested (8-10 days)).

Non-pathogenic for guinea pigs.

1. Tyrosine crystals not observed.

Clostridium sporogenes

2. Tyrosine crystals observed.

Clostridium tyrosinogenes

- (b) Indol /, Nitrate -, Gelatin /, Motility /, Nagler /.

Glucose /, Lactose -, Sucrose -, Salicin /.

Spores ovoid, abundant, central to excentric, not markedly if at all swelling rod.

Pathogenic for guinea pig.

Clostridium sordelli

Non-pathogenic for guinea pig.

Clostridium bifermentans

Clostridium centrosporogenes

Lead Acetate: no blackening, no browning.

Nitrate -, Indol -, Gelatin /, Motility /.

(Wine red color in iron gelatin (24-48 hours)).

Glucose -, Lactose -, Sucrose -, Salicin -.

Spores ovoid, abundant, excentric to sub-terminal, swelling rod, micro-aerophilic.

Pathogenic for guinea pig (produces extensive tissue lysis).

Clostridium histolyticum

5. Iron Milk: no gaseous fermentation, no digestion, no blackening, coagulation late if any (15-20 days or more).

Lead Acetate: not blackened, but showing smoky browning at 24-48 hours, not measurably increased on incubation.

Nitrate -, Indol $\frac{+}{-}$, Gelatin $\frac{+}{-}$, Motility $\frac{+}{-}$, Nagler -.

Glucose -, Lactose -, Sucrose -, Salicin -.

Spores spherical, not abundant, terminal, swelling rod.

Pathogenic for guinea pig.

(1) Toxic

Clostridium tetani

(2) Non-toxic

Clostridium putrificum

Lead Acetate: blackened

Nitrate -, Indol -, Gelatin -, Motility $\frac{+}{-}$, Nagler -.

Glucose -, Lactose -, Sucrose -, Salicin -.

Spores oval, terminal, swelling rod.

Non-pathogenic for guinea pig.

Clostridium cochlearium

6. Iron Milk: inactive gaseous fermentation, slow coagulation, no digestion, blackening or browning not recorded.

Lead Acetate: probably blackened.

Nitrate not recorded, Indol not recorded, Gelatin $\frac{+}{-}$, Motility $\frac{+}{-}$.

Glucose $\frac{+}{-}$, Lactose $\frac{+}{-}$, Sucrose -, Salicin $\frac{+}{-}$.

Spores rare, ovoid, sub-terminal, slightly swelling rod.

Pathogenicity - Slight for guinea pig.

Clostridium aerofaecium

Results: Using these technics on material obtained from war wounds or amputated extremities, the results tabulated in Table XXXVII were obtained.

Acrolein Test for Cl. perfringens - A medium derived from that proposed by Humphreys (1) has been employed for detection of formation of acrolein aldehyde. The formula follows Table XXXVII.

Table XXXVII. Anaerobic Culture of War Wounds from Korea

<u>Clostridium novyi</u> (pathogenicity doubtful).....	1
<u>Clostridium novyi</u>	2
<u>Clostridium sporogenes</u>	21
<u>Clostridium bifermentans</u>	15
<u>Clostridium putrificum</u>	19
<u>Clostridium tertium</u>	2
<u>Clostridium cochlearium</u>	6
<u>Clostridium fallax</u>	1
<u>Clostridium filiforme</u>	1
<u>Clostridium butyricum</u>	1
<u>Clostridium tetani</u>	4
<u>Clostridium capitovale</u>	4
<u>Clostridium perfringens</u>	47
<u>Clostridium perfringens</u> (non-pathogenic)	2
<u>Clostridium carnis</u>	1
<u>Clostridium sphenoides</u>	1
<u>Clostridium aerofaecitidium</u>	1
<u>Clostridium septicum</u>	2
<u>Clostridium sordelli</u>	1
<u>Clostridium tetanomorphum</u>	2
<u>Clostridium</u> , species undetermined	6
No <u>Clostridium</u> recovered	27

Thioglycollate Glycerol Medium:

Yeast extract (10% solution)	50.0 cc.
Proteose-peptone	1.5 gm.
Sodium chloride	2.5 gm.
d-cystine	0.05 gm.
Sodium thioglycollate	0.5 gm.
Glycerol	20.0 cc.
Agar	0.75 gm.
Bromcresol purple solution	7.5 cc.
Dispense in screw cap bottles in 100 cc. amounts. Bottles should be filled to keep air space to a minimum.	
Autoclave 15 lbs. 15 minutes.	

The principal alteration from the formula advocated by Humphreys et al. is the substitution of sodium thioglycollate for ascorbic acid and the addition of cystine and yeast extract to the medium.

At the time of testing, a portion of glycerol broth sufficient to complete all tests at hand is transferred to culture tubes, which are heated in the boiling water bath for 10 minutes, then rapidly cooled. 0.1 cc. of the culture supernate from cooked meat broth is placed in a sterile serological tube, 2 cc. of the freshly heated and cooled medium is added, and the tubes are incubated at 37°C. Fermentation of the glycerol is indicated by shift of the medium to an acid reaction. At 6 hours incubation, one cc. of broth is transferred to another tube; to it is added one cc. of freshly acidified Schiff's reagent. Appearance of a deep red or purple-red color indicates the presence of acrolein. Cultures negative at 6 hours are retested at 24 hours incubation. A control culture of Cl. perfringens is included in the test run.

Media Production - This usually unnoticed but vital section of a Bacteriology department frequently serves as a good index of the activity of the entire department. During the year this group prepared and dispensed 100 different types of media in the following amounts:

Plates	43,366
Slants	56,363
Tubes	90,199
Bulk media	546 cc.
Total liters of media	6,429

Diagnostic Biologics Production - Although most diagnostic biologicals are procured from the AMSGS for distribution to other laboratories in the FEC and for use in this laboratory, certain such agents are also produced locally. To meet the needs the Enteric Section of this department, and as a training method to provide a more comprehensive understanding of the field diagnostic sera are produced for the Shigella. These included the following:

Group Sera: Polyvalent A	144
Polyvalent B	60
Polyvalent C	60
Specific, single factor sera	5781
Experimental Sera (Group)	<u>804</u>
Total <u>Shigella</u> Sera	6849 cc.

DEPARTMENT OF CHEMISTRY

The Department of Chemistry is divided into six functional sections, which serve the Far East Command in the capacity of a general chemical laboratory. Due to an increased demand, sections of Food Chemistry and Allergy investigation have been added during the last quarter of the reporting year.

Table I shows a breakdown of work submitted to the various functional sections of the Department of Chemistry:

Table I. Routine Chemical Examinations

<u>Section</u>	<u>Specimens Submitted</u>			<u>Determinations Completed</u>		
	<u>1949</u>	<u>1950</u>	<u>Increase (%)</u>	<u>1949</u>	<u>1950</u>	<u>Increase (%)</u>
Clinical Chemistry	3860	4177	8.22	5593	6438	15.10
Water Analysis	168	259	53.57	3978	4923	23.73
Toxicology	357	621	74.17	1660	4449	167.88
Food Chemistry	---	(179*)	---	---	(388*)	---
Allergy Investigation	---	198	---	---	1210	---
Miscellaneous Analysis	1461	3560	144.44	2743	6685	143.71
Totals	<u>5846</u>	<u>8815</u>	<u>50.82</u>	<u>13,974</u>	<u>23,705</u>	<u>69.15</u>

* Not included in grand total, since these figures are included for purposes of calculation, as a part of Miscellaneous Analysis Section.

CLINICAL CHEMISTRY: The Clinical Chemistry Section operates as a diagnostic service to medical installations of all components of the Armed Forces within the Far East Command. Due to the fact that most of these organizations have laboratory facilities for performing most routine examinations, the bulk of the work of the Clinical Chemistry Section consists of those types of determinations requiring either special techniques, special apparatus, or other facilities not available to the less completely equipped unit laboratories.

The following table lists specimens received in the Clinical Chemistry Section during 1950:

Table II. Clinical Specimens Received in 1950

<u>Specimen</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>	<u>Dec</u>	<u>Total</u>
Blood	221	176	124	144	232	174	150	120	124	134	164	172	1935
Feces	18	10	7	6	32	15	9	30	6	10	17	13	173
Serum	61	96	99	125	123	139	138	154	64	59	70	181	1309
Spinal Fluid	46	64	73	72	44	27	15	49	36	32	30	44	532
Urine	6	1	7	6	10	12	22	32	40	25	20	26	207
Others	-	-	2	3	5	5	-	-	1	3	2	-	21
Combined Total													<u>4177</u>

In order to give the reader a more complete perspective of the type of determinations requested of this laboratory, Table III lists determinations and number of each completed during the past year.

It is the policy of the Clinical Chemistry Section to appraise current developments and newly published methods and to adopt these methods if they prove to be more accurate and practical than the methods currently in use. For example, the Biuret method (1), using albumin as a standard for protein determination, is being set up to replace the more time consuming and less accurate Tyrosine procedure (2).

Table III. Determinations Performed in Clinical Chemistry

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
A/G Ratio	130	Bilirubin	327
Albumin	132	Bence Jones Protein	1
Alcohol, ethyl	670	Calcium	55
Amylase	59	Calculi	7
Bile	16	Cephalin Flocculation ..	1237
Bromosulphalein	38	Carbon Dioxide	9
Chlorides	68	Magnesium	1
Cholesterol, Total	256	Non-Protein Nitrogen ...	144
Cholesterol, Esters	89	Occult Blood	160
Clot Retraction	1	Phenolsulfathalein	4
Creatine	1	Phosphatase, Acid	41
Creatinine	22	Phosphatase, Alkaline ..	417
Fat (Qual.)	7	Phosphorus	45
Fat (Quan.)	2	Potassium	66
Fatty Acids	1	Porphyryns	9
Fibrogens	2	Protein, Total	599
Galactose	1	Prothrombin	10
Gastric Acidity	2	Sodium	59
Globulin.....	129	Starch	1
Globulin (Qual.)	302	Sulfa Level	5
Glucose	294	Salicylates	5
Glucose Tolerance	16	Trypsin	8
Icteric Index	137	Urea Clearance	3
17-ketosteroids	156	Urea Nitrogen	46
Lead	3	Uric Acid	36
Lipase	9	Urobilinogen	5
Lipids	1	Vitamin C	4
		Total	6438

Evaluation of the cadmium sulfate liver function test (3) was undertaken. During a 30 day period, all sera submitted for cephalin cholesterol flocculation and thymol turbidity tests were subjected to examination by the cadmium sulfate method. Results were erratic and no correlation could be established between the three determinations.

The determination of 17-ketosteroids by the method Landau (4) was adopted. A special project involving 17-ketosteroids determinations was begun in conjunction with the medical service of the 155th Station Hospital to study the effect of ACTH administration to patients having a clinical diagnosis of infectious hepatitis. Both pre-treatment and post-treatment specimens were submitted on selected patients. Results were varied and little correlation could be established. Of 56 patients examined, 14 did not have two specimens submitted, 17 pairs showed an increase, 10 showed a decreased response, and 15 remained unchanged after treatment. However, of the 56 patients examined, 24 had a 17-ketosteroids value of less than 12 milligrams percent (normal 12-28 for males).

An attempt was made to institute the blood iodine method of Connor (5). An iodine still was prepared by Japanese manufacturers but misfortune was encountered in the fragility of the apparatus, and difficulty in procuring reagents of suitable purity. As yet, this determination is not sufficiently well established to be offered by this laboratory.

A method of Kingsley and Schaffert (6) for determination of cholesterol was investigated. It was decided that despite the shorter working time employed by the Kingsley-Schaffert method (two hours as opposed to 24 hours by the Sperry-Schoenheimer method),

the techniques of the procedure were too involved and exacting to be of practical use.

The zinc-uranyl acetate method for sodium, as described by Stone and Goldzieher (7) was evaluated and found unsatisfactory for application to the Coleman spectrophotometer, due to the flat curve obtained with higher concentrations of sodium. A gravimetric method utilizing zinc-uranyl acetate (8) is being used presently and is found to be very satisfactory.

For such determinations as cholesterol esters, creatinine and phosphatase, SOP's had previously been set up to allow direct reading spectrophotometrically. The transitory nature of color developed in these reactions, and the variation of individual techniques, requires processing a standard with each determination rather than relying on a graph for calculation of results.

TOXICOLOGY: The Chemistry Department maintains a Toxicology Section which, with the progress of time, is improving its methods and techniques by the addition of new apparatus and by adopting procedures which have been demonstrated to be an improvement over the methods previously used.

Since the preponderance of material submitted is from autopsy materials (171 autopsies in 1950), in cases where cause of death is obscure, the methods employed must necessarily be such that positive results will leave no question concerning validity and accuracy. Members of the Department of Chemistry and Department of Pathology have been called upon to act in the capacity of expert witnesses for Courts Martial.

Table IV indicates the number and type of specimens received, while Table V shows the various examinations that have been required during the course of toxicological examinations this past year.

A brief review of the significant positive findings is given below:

Ethyl Alcohol - This substance was found in significant concentrations in 40 per cent (69 cases out of 171) of the autopsies completed during the year. This is a considerable increase over the 23 per cent in 1949.

Chlorides - Comparison of chlorides in blood from right and left ventricles was made in fourteen cases of suspected drowning.

Barbiturates - Positive quantitative results were obtained for barbiturates in fourteen cases during the year.

Paraldehyde - This substance was detected in the tissues of Case No. 3703 in the following concentrations: Liver - 7.0 mg%, kidney - 2.6 mg%, lung - 1.4 mg%.

Chloral Hydrate - One autopsy (No. 1301), which showed high ethyl alcohol concentration in tissues and stomach, also gave positive results for chloral hydrate in stomach contents.

Heavy Metals - Mercury was detected in the kidney and liver of one case (No. 1569). Quantitative determination showed the concentration to be 0.15 mg. per 100 grams of kidney and 0.02 mg. per 100 grams of liver.

Carbon Monoxide - This substance was found in 4 autopsy cases:

Case 835 - 77.5% blood saturation
Case 1408 - 85.0% blood saturation
Case 1221 - 45.0% blood saturation
Case 4877 - 60.0% blood saturation

Alkaloids - Six autopsy cases gave positive results for alkaloids. In 5 cases (1832, 1838, 1839, 1878, 2257) the alcohol-chloroform extract produced positive color and precipitation reactions, and the alkaloid was placed in Group II, Bamford's Classification (9).

Table IV: Toxicology Specimens Received

<u>Tissue or Fluid</u>	<u>No.</u>	<u>Tissue or Fluid</u>	<u>No.</u>
Blood	153	Lung	3
Brain	85	Peritoneal Fluid	1
Heart	3	Spleen	1
Intestinal Contents ..	1	Stomach Contents	116
Kidney	45	Tissue, Mixed	13
Kidney Pus	1	Urine	77
Liver	121	Vomit	1
		Total	621

Table V. Toxicological Examinations Completed

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
Alcohol, Ethyl	564	Magnesium	28
Alcohol, Methyl	392	Mercury	3
Aldehydes	315	Metals, Heavy	89
Alkaloids	646	Mineral Acids	3
Amphetamine	12	Morphine	409
Arsenic	1	Non-protein Nitrogen ..	10
Barbiturates	395	Paraldehyde	4
Benzedrine	2	Phenols	611
Bilirubin	2	Phosphorus	4
Calcium	1	Picrotoxin	1
Carbon Dioxide	3	Potassium	1
Carbon Monoxide	9	Protein	2
Chloral Hydrate	18	Pyribenzamine	14
Chlorides	38	Salicylates	3
Creatinine	1	Sodium	3
Cyanides	326	Sugar	4
Formaldehyde	181	Sulfa Drugs	12
Halogenated Compounds .	327	Strychnine	1
Heroin	6	Urea Nitrogen	6
Iodine	1	Van den Bergh	1
		Total	4449

In these five cases, the preponderance of evidence indicated a morphine derivative as having been taken by the deceased. In one case (4653), the alkaloid was positively identified as morphine.

One autopsy case (1021) concerned possibility of death of a child due to pyribenzamine sensitivity. This laboratory had no satisfactory method for positive identification of pyribenzamine and chemical spot tests failed to identify the material. A picric acid derivative was prepared and forwarded to the Army Medical Service Graduate School Laboratory for microscopic analysis. There, positive identification could not be made, due to the small amount of the unknown present but it was not felt to be pyribenzamine. Since only a very small amount of stomach contents and tissues was submitted, complete isolation of the toxic agent was not possible; however, using the method described in this report under the heading of "Investigation of Methods", positive results were obtained for an organic base not common to the body, showing 340 micrograms per gram of stomach contents and 20 micrograms per gram of liver.

FOOD CHEMISTRY: The Food Chemistry Section has been added as a result of the outbreak of hostilities in Korea, which required the Quartermaster to develop a field type ration for both South Korean and Turkish troops. Only limited space requirements are available for chemical assay of food; however, the section has been able to satisfactorily set up determinations for protein, fat, carbohydrate, moisture, ash, and calculation of caloric content. Methods used are essentially those of Standard Methods of Analysis of the Association of Official Agricultural Chemists (AOAC), 6th Edition, 1945, with other standard texts being used if necessary. Vitamin assay and prolonged incubation tests are not presently possible due to lack of proper laboratory instruments and the limitations dictated by working space available. Samples analyzed and types of determinations run on the submitted samples are shown in Table VI below.

Table VI. Food Chemistry Determinations

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
Ammonia	1	Methyl Chloride	4
Artificial Coloring	1	Moisture	8
Ash	4	Nitrogen	1
Butter Fat	86	pH	24
Calculation of caloric value .	1	Potassium	1
Carbohydrate	3	Protein	5
Cellulose	1	Sediment	53
Chlorine	1	Sodium Chloride	1
Fat	6	Solids not fat	86
Fiber	1	Starch	1
Heavy Metals	4	Toxicity Test (ad libitum) ...	8
Iodine	1	Total Solids	86
		Total	388

Remarks - The analysis of dairy products was carried out as an aid to control the quality of recombined milk products by processing plants in Japan. Of 53 white milk samples received, 8 (15.1%) were low in butter fat or total solids. Of 42 chocolate milk samples received, 5 (11.9%) were low in butter fat content, and of 32 ice cream mix samples submitted, 3 (9.3%) were low in butter fat content. Until the latter half of the year, the contract company was relying on the Babcock method of butter fat determinations, a method held not applicable to recombined milk. Gottlieb's method (10) is standard for this laboratory. After adopting the new method, all milks now apparently are sufficiently controlled to assure their meeting contract requirements.

Miscellaneous analyses concerning food consisted of pH determinations on shrimp, oysters, and scallops, and toxicity tests on suspected samples of canned beets, boysenberries, olives, and rice balls. In two cases, stored meats were suspected of being contaminated with ethyl chloride refrigerating agent or ammonia, but on examination were found to be innocuous. For preparation of a ration, ROK, combat, the Quartermaster referred samples to this laboratory for food analysis and calculation of caloric value. Other samples of rations developed were submitted, but analysis was not completed at the time of the compilation of this report.

WATER ANALYSIS: The Water Analysis Section received 259 specimens during the year. Of these, 179 were potable, 70 boiler, 1 combined potable and boiler, 8 for silicate analysis, and 1 for determination of alkalinity and acidity only. As a guide to the variety of determinations required for processing of these samples (11), the number and type of determinations are listed as Table VII. The service of this laboratory does not necessarily pertain to medical department functions alone, since special problems of the Government Agencies within the Far East Command have been considered.

Samples were received at a rather steady rate of 10 per month, with the exception of August and October. In August, samples of pyrogen-free water, prepared by the Bacteriology Department of this laboratory, were submitted to assure adequate control in producing a water of less than 10 parts per million total solids (12). Also that month

Table VII. Determinations of Water Analysis

<u>Determination</u>	<u>No.</u>	<u>Determination</u>	<u>No.</u>
Acidity, methyl orange	3	Manganese	168
Acidity, phenolphthalein	3	Nitrogen, ammonium	126
Alkalinity, caustic	18	Nitrogen, nitrate	147
Alkalinity, methyl orange ...	223	Nitrogen, nitrite	146
Alkalinity, phenolphthalein .	223	Odor	240
Aluminum	6	pH	213
Calcium	213	Residue, dissolved	224
Chloride	221	Residue, fixed	226
Color	197	Residue, suspended	230
Hardness (calc. from Fe, Ca		Residue, total	315
and Mg	92	Sediment	184
Hardness (calc. as non-car-		Silica	240
bonate)	49	Sodium and Potassium	88
Heavy Metals	4	Specific Gravity	3
Iron	7	Sulfate	206
Iron & Aluminum	213	Taste	234
Lead	18	Turbidity	231
Magnesium	212		
		Total	4923

saw numerous samples submitted by Far East Air Material Command for total solids check on both distilled water and regular water sources of Korea and Japan. This was done to provide suitable water for use in injection type engines and airplane cooling systems. This project has been carried out through the balance of the year and will continue as long as required.

One special problem of analysis was encountered by an Aircraft and Warning Squadron. They found that aluminum tanks used by their unit corroded badly. Water analysis showed a water of high pH with large amounts of iron, magnesium, sulfate, and carbonate present, which suggested that the aluminum, by virtue of its greater electrical potential, was being corroded by the water of high iron content (13). Also, the high carbonate and sulfate values were indicated as causing corrosion of aluminum tanks. Since advice in preventing corrosion was solicited, the use of the soda-lime process of softening water (14) was advocated as an expedient. It was also suggested that the tanks be replaced by stainless steel ones, and this was done. No further problems have been noted from this unit.

ALLERGY INVESTIGATION: With the assignment of a competent botanist in September, the Allergy Investigation Section was added to the Chemistry Department. Preliminary work consisted of collection of literature and data concerning allergy conditions among the Japanese, the most conspicuous findings being the limited publications concerning allergies in Japan.

This laboratory facility was initiated as a result of conference with the medical staff of the 155th Station Hospital, which established the existence of a type of asthma among occupation personnel. Asthmatics of this type apparently are localized within the vicinity of Yokohama and further restricted to the season of the year between the months of September and February. Symptomatology is that of true asthma; however, the usual drugs employed in the treatment of allergic asthmas are without value. The incidence of the disease is high, with approximately ten percent of patients seen on emergency status being those manifesting symptoms of asthma. Many of these cases observed were reported completely relieved of symptoms when placed outside Yokohama on leave status, but experienced relapse when returning to their Yokohama stations.

A chronology of the pollinating plants of the Tokyo-Yokohama areas has been prepared. Approximately 500 pollinating plants have been collected and reference slides prepared from them to be used in further investigations. With the cooperation of the laboratory at the 155th Station Hospital, daily slide collections of atmospheric particles have been made, using the method recommended by American Academy of Allergy (16). Examinations of these slides and comparison of the Yokohama collections with those of the Tokyo vicinity has not yet offered any distinct variation between the two collections. A spot map locating living and work areas of patients in Yokohama is being prepared, with the hope that it will show some concentration of incidence within specific areas and thus give a lead for further investigation. Air pollution by industrial fumes may well be a factor.

With the assignment of a competent technician in December, Veldee's methods for collection of pollens (17) was instituted. From the separated pollens, antigens will be prepared according to the method of Strauss (18). To date approximately 20 sterile antigens have been prepared and many more are forthcoming.

For the coming year, this section will continue above studies with clinical testing of cases with allergens. In addition, the culture of molds and fungi in the Yokohama area, collected by the plate culture method outlined by Durham (19) will be accomplished.

MISCELLANEOUS ANALYSIS: Source of these varied samples has included all types of organizations in the Far East Command. For purpose of operation, certain types of examinations done routinely are included in the miscellaneous classification and examination of Table VIII will give the reader an idea as to the diversity of samples submitted.

Table VIII. Samples Received for Miscellaneous Analysis Section

<u>Sample</u>	<u>No.</u>	<u>Sample</u>	<u>No.</u>
Alcohol, ethyl	22	Kerosene	1
Alcoholic Beverages	87	Leaves and Flowers	1
Bacteriological Media	29	Mikroklene, antiseptic rinse.	3
Body Fluids	125	Mosquito, dehydrated	2
Boiler Residue	1	Nitrous Oxide	1
Calcium Hypochlorite	31	Oxygen	656
Cigarettes	1	Pharmaceuticals	16
Dairy Products	86	Porpoise Milk	1
DDT	15	Procaine Hydrochloride	2
Ethylene Glycol	1	Prophylactics	288
Food Products	47	Santobrite	1
Halazone Tablets	1946	Soap	37
Hydrogen Peroxide	24	Sulfuric Acid	1
Ice	1	Unknown Substances	62
Insect Repellent	2	Water	70
		Total	3560

The determinations of this section of Chemistry may be divided into the two main subheadings of qualitative and quantitative analysis, with toxicity tests and determination of physical constants playing minor roles. An appraisal of the variety of tests required of this section and the number of reagents required may be made by examining Table IX which lists the variety of examinations done in making identifications and complying with the requests submitted with samples.

Work included assay of DDT, analysis of chemicals for requirements according to USP standards, checking of mechanical prophylactics to assure a reliable product, assay of numerous beer and alcoholic beverage samples to assure proper quality, examination of liquors for poisonous substances, toxicity tests on numerous solutions and food products, checking of some bacteriological media, assay of fat content of mosquito samples for Entomology Department, checking of a sample of improperly labeled nitrous oxide which proved contaminated with carbon dioxide, and other analyses of a similar nature.

Table IX. Determinations Completed by Miscellaneous Analysis Section

<u>Qualitative</u>	<u>No.</u>	<u>Qualitative</u>	<u>No.</u>
Acids	129	Marihuana	4
Aconitine	12	Meconic Acid	2
Alcohol, ethyl	10	Mercury	1
Alcohol, methyl	74	Merthiolate	4
Aldehydes	94	Morphine	166
Alkali Carbonates	1	Nickel	2
Alkalies	129	Nitrates	14
Alkaloids	169	Nitrites	12
Aluminum	2	Non-volatile Substances	5
Amino Acids	2	Organic Matter	8
Ammonia	2	Oxidizing Substances	116
Ammonium Nitrogen	1	Phenol	79
Aromatic compounds	1	Phenacetin	1
Arsenic	8	Phthalic Acid	1
Artificial coloring	8	Picrotoxin	2
Asorbic Acid	3	Platinum	1
Aspirin	7	Pontocaine	1
Barbiturates	99	Potassium	1
Barium	6	Potassium Ferrocyanide	1
Benzedrine	25	Preservatives	6
Benzoates	2	Procaine Hydrochloride	24
Benzoic acid	1	Pyribenzamine	6
Blood	4	Reaction to Bromine	1
Bromine	1	Reducing Sugars	17
Bromural	2	Resin	4
Caffeine	10	Rosin	3
Calcium	13	Rotenone	2
Calcium Carbonate	3	Salicylates	3
Calcium Hypochlorite	3	Silica	1
Carbonate	5	Silicate	1
Carbon Dioxide	117	Soap	1
Carbonizable matter	1	Sodium	9
Carbon Monoxide	123	Spectrographic analysis	2
Carnauba Wax	5	Spermatzoa	2
Cellulose	1	Starch	6
Chloral Hydrate	72	Sugars	1
Chlorides	8	Sulfonamides	2
Cholesterol	1	Sulfates	2
Cobalt	3	Sulfathiazole decomposition	12
Cod Liver Oil	1	Sulfur	1
Copper	16	Thiamine	6
Coriander Oil	1	Tin	1
Cyanides	68	Ureides	3
Diethylstilbesterol	2	Yohimbine	1
Dye	1	Zinc	5
Flourides, inorganic	3		
Flourides, organic	1	Total	2193
Formaldehyde	5		
Free Chlorine	2	<u>Quantitative</u>	<u>No.</u>
Fusel Oil	74	Acidity	10
Grease	2	Alcohol, ethyl	113
Halogens	128	Alcohol, methyl	21
Heavy Metals	89	Alkali Carbonates	3
Heroin	9	Alkali Hydroxides	3
Hydroxides	1	Alkalinity	1
Insulin	1	Aluminum	1
Iodine	1	Anhydrous Soap	22
Iron	13	Ash	5
Ketones	71	Atropine	1
Lead	1	Barbiturates	4
Maltose	3	Benadryl	2
Manganese	1		

Table IX. Continued

<u>Quantitative</u>	<u>No.</u>	<u>Quantitative</u>	<u>No.</u>
Benzoic Acid	1	Nitrogen	3
Butter Fat	111	Non-volatile residue	7
Calcium	3	Organic Matter	2
Calcium Hydroxide	1	Oxygen Assay	655
Calorie Determinations	3	Oxidizing Substances	1
Carbohydrates	4	Phosphates	1
Chlorides	40	Phthalic Acid	1
Chlorine, available	2321	Pontocaine	1
Combined Alkali	2	Potassium	1
DDT Assay	8	Potassium Hydroxide	1
Ether Extract	4	Procaine	5
Fat	5	Protein	8
Fiber	4	Residue on ignition	1
Fixed Residue	1	Residue, insoluble	4
Free Acid	20	Rosin	18
Free Alkali	19	Sediment	46
Free Carbonate	3	Silicates	3
Hydrogen Peroxide	5	Sodium	2
Insulin Assay	1	Sodium Carbonate	5
Iron	3	Sodium Chloride	5
Lactic Acid	1	Sodium Hydroxide Assay	1
Lead	42	Solids not fat	38
Loss on drying	1	Sulfanilamide Assay	1
Matter insoluble in alcohol	23	Sulfate	1
Matter insoluble in water	27	Sulfathiazole Assay	12
Matter volatile at 105°C.	22	Sulfuric Acid Assay	1
Merthiolate Assay	1	Starch	1
Moisture	8	Titratable Acidity	1
		Total Solids	115
		Water	9
		Total	3821

<u>Physical Constants</u>	<u>No.</u>	<u>Miscellaneous Determinations</u>	<u>No.</u>
Boiling Point	2	Inflation Retention Check	149
Color	9	Measurement for Specifications	149
Distillation	9	Microscopic examination of	
Melting Point	30	Plant Fibers	2
Odor and Appearance	17	Preparation of Solutions	5
pH	55	Preparation of Specific Grav-	
Reaction	2	ity Chart	1
Refractive Index	11	Preparation of Temperature	
Solubility	18	Change Chart	1
Specific Gravity	38	Rabbit Insulin Assay	10
Total	191	Toxicity Tests, Fish	5
		Toxicity Tests, Mice	158
		Total	480

Total Miscellaneous Determinations - 6,685

The variety of unknowns submitted for identification, often with the statement "For Identification" as the only lead, has led to a considerable amount of chemical detective work. The method found most advantageous by this laboratory, in identifying unknowns, is as outlined by Feigl (20). Huntress and Mulliken (21) has also been very valuable in identifying organic compounds. The use of laboratory animals has become routine in establishing toxicity of specimens submitted. Of 62 samples accepted to be worked on

and identified, the Department of Chemistry has experienced good success in complying with the request. Identifications have included rat poison (ANTU), benzedrine, bromural, thiamine hydrochloride, insulin, ethyl alcohol, ascorbic acid, maltose, heroin, carnauba wax, lubricating grease, copper carbonate, alkaloids, potassium ferrocyanide, opium, morphine, pyribenzamine, calcium chloride, assay of GU-cide (a proprietary North Korea drug) which proved to be a mixed tablet of sulfanilamide and sodium bicarbonate, and other miscellaneous identifications.

Probably the most unusual request was for analysis of a sample of porpoise milk by Natural Resources Section, GHQ. This product showed 2.21 percent fat, 66.2 percent moisture, and 31.6 percent protein. One project involving a series of "classified" specimens required a great deal of work and a wide investigation involving many chemical methods. It was gratifying to see the results of this laboratory confirmed by the Biological Department, Chemical Corps, Camp Dietrick, Maryland.

Three critical items for the Air Force (FEAMCOM Procurement Section) were assayed for the necessary specification requirements. Sulfuric acid for batteries was checked to provide an emergency purchase of a product of satisfactory purity. Samples of ethyl alcohol, denatured, were analyzed for impurities and other specification checks. Using these laboratory tests as a basis (of 22 samples examined, 5 were unsatisfactory), the agency concerned was able to procure alcohol of sufficient quality in adequate amount to meet their demands.

Some miscellaneous projects were undertaken by the Chemistry Department. One of these involved the procurement of oxygen for Medical Department use. The logistical problem posed by transportation of large numbers of oxygen cylinders from the Yokohama Medical Depot to hospitals in Korea and South Japan required procurement of oxygen locally for these areas. Oxygen producing plants were located by the Supply Division, Eighth Army, and this laboratory furnished two teams of one officer and one enlisted technician each to assay oxygen (22) at source and provide for the training of competent technicians to continue the work. Both teams accomplished their mission and returned to the base laboratory. The problem of equipping these outlying laboratories was carried out by the Chemistry Department by special purchase from local Japanese firms, since regular supply channels could not furnish the necessary equipment and reagents on such short notice.

The Chemistry Department assisted the Sanitary Engineer, Medical Section, and Transportation Section, GHQ in recommendations for processing ships, which had previously carried leaded gasoline and oil, to render them safe for transportation of fresh water to be used by troops during amphibious operations in Korea. It was recommended that tanks be cleaned by washing with soap and water, followed by a thorough flushing with steam, final processing to consist of either sand blasting or the use of cement wash. This project was carried out and a check on procedure was done by the Chemistry Department. Using the method of digestion of Callingaert and Gambrill (23) and the Dithizone titration method of lead analysis (24), specimens were examined from tankers before and after processing. It was requested that the tanks be flushed with water and a sample of this flushing be submitted as a check before processing and for the final sampling to be carried out by filling the tank with water and allowing it to stand for 48 hours. Request was made to collect all samples in hard glass bottles, cleaned and furnished by this laboratory; however, not all samples were submitted in the proper containers and since the apparatus available would not handle crude oil samples for digestions, the samples consisting of only crude oil could not be satisfactorily analyzed. Final samples submitted, however, were quite satisfactory, with one exception.

The Chemistry Department has been called upon frequently to determine the amount of available chlorine in Halazone tablets. While the chlorine in Halazone (p-toluolsulfon-dichloramino-benzoic acid) is more stable than that found in inorganic combination (e.g. calcium hypochlorite), chlorine loss occurs on long standing, particularly if improper sealing of containers permits exposure to atmospheric moisture. It is therefore necessary that Halazone tablets, stored for long periods, be checked before they are used. At the onset of the Korean crisis and for several weeks thereafter, this laboratory received what at first appeared to be more Halazone tablets than could be handled with

facilities available in the short time required by the emergency. By modifying the more lengthy procedure given by the National Formulary (26) it was decided that analysis of these samples could be accomplished rapidly, without sacrificing accuracy appreciably. A total of 958 sample bottles of Halazone tablets, representing 178 lots, were analyzed for available chlorine, using the modified method, as follows:

Reagents -

1. Sodium hydroxide, 10%.
2. Potassium iodide, 15%.
3. Acetic Acid, 35%.
4. Standard sodium thiosulfate, 0.02 N.
5. Standard potassium dichromate solution, 0.1000 N.
6. Soluble starch solution, 1%.
7. Hydrochloric acid, concentrated.

Preparation of Standard Solutions -

1. Standard potassium dichromate.
 - a. Dissolve exactly 4.093 grams of the pure, dry salt in water and dilute to 1 liter.
2. Standard sodium thiosulfate solution.
 - a. Stock solution; dissolve 25 grams of the salt in water and dilute to 1 liter.
 - b. Working solution: Dilute 200 ml. of the stock solution to 1 liter and standardize against standard potassium dichromate solution. The solution should be standardized before each daily series of analyses.
 - c. Standardization of thiosulfate solution: To 3 ml. of standard potassium dichromate contained in an Erlenmeyer flask, add 5 ml. of water, 1 ml. of HCl, and 2 ml. of potassium iodide solution. Stopper and allow to stand in the dark for 5 minutes. Add 20 ml. of water and titrate with the working thiosulfate solution. When the yellow color has almost disappeared, add 0.5 ml. of the starch solution and titrate to the disappearance of the blue color.

$$\text{Calculation: } \frac{0.3}{\text{ml. of thiosulfate}} = N \text{ of thiosulfate}$$

Procedure - Place three whole, undecomposed Halazone tablets in a 50 ml. Erlenmeyer flask and add 7 ml. of water and 1 ml. of sodium hydroxide. Break up the tablets with a stirring rod and shake until solution is effected. Add 1 ml. of the potassium iodide solution and 1 ml. of acetic acid and titrate immediately with standard thiosulfate solution. Add 0.5 ml. of starch solution near the end of the titration to establish the end point.

Calculation - One ml. of 0.1 thiosulfate is equivalent to 1.773mg. available chlorine. Therefore, calculation of available chlorine per tablet may be made by use of the following formula:

$$\text{ml. of thiosulfate} \times \frac{\text{Normality thiosulfate}}{0.1} \times 1.773 \times \frac{1}{\text{No. of tablets used}} \\ \text{equals mg. available chlorine per tablet.}$$

INVESTIGATION OF METHODS AND RESEARCH: Barbiturate Analysis - The Chemistry Department is frequently required to assay body fluids and tissues for their barbiturate content. Existing methods for such determinations have not proven satisfactory. Not only have the actual chemical methods proved disappointing, but published procedures for the extraction of the barbiturates from tissues and fluids have given low yields or have failed to exclude substances which interfere with the final reaction. By modifying and improving the procedures given in the literature, an attempt has been made to develop a reliable quantitative method for the determination of barbiturates in body fluids and tissues applicable to routine toxicological and clinical analysis. The investigational work was started by Lt. Balikov in 1948 (27) and the progress made during 1949 has been reported (28).

The determination is based on the observation (29, 30, 31) that barbiturates produce a purple colored complex with anhydrous cobaltous ion in the presence of an alkaline agent. Koppanyi (32) adapted this reaction to the quantitative determination of barbiturates, and it is essentially his methods that were applied in this laboratory.

To insure reproducible results and to facilitate the ease with which the reaction can be carried to completion, it was found necessary to introduce several modifications of Koppanyi's method. These can be listed briefly as follows:

1. Due to difficulty encountered in obtaining absolutely dry methyl alcohol, chloroform was substituted for the alcohol in preparing the isopropylamine reagent.
2. Since it is more readily dried, cobaltous chloride was substituted for cobaltous acetate.
3. In making up the stock cobalt solution, alcohol was added to increase the solubility of cobaltous chloride.
4. Instead of reading the color intensity immediately after the two reagents had been combined, the reading was taken ten minutes later. In this manner, maximum color development was obtained. It was also found necessary to employ a reference reagent blank.

Reagents -

1. Standard aqueous cobaltous chloride solution: 3.64 gram of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ is dissolved in water and made up to 100 ml. This is equivalent to a 2% CoCl_2 solution. The solution can be standardized by the gravimetric alpha-nitroso-beta-naphthol method (33), but experience has shown that this is unnecessary if the hydrated crystals from a well-capped bottle are weighed accurately.
2. Preparation of stock alcoholic CoCl_2 solution: Approximately 4 grams of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ are dried in an evaporating dish over a small open flame. The dry mass is pulverized and made up immediately to 25 ml. with dehydrated ethyl alcohol. Stopper and shake vigorously until a dark blue solution is obtained. Allow 15 minutes for the excess CoCl_2 to settle out. Place two and three ml. aliquots of the supernatant in 50 ml. volumetric flasks and make up to the marks with water. Shake and read the transmittance percentage on a Coleman Junior Spectrophotometer at 510 m μ , using water as the reference. Calculate the concentration of CoCl_2 from the C-T curve obtained by reading dilutions of the standard aqueous CoCl_2 solution.
3. CoCl_2 chloroform reagent (1/25 M.): A measured volume of the alcoholic CoCl_2 stock solution, sufficient to make 50 ml. of a 1/25 M CoCl_2 solution, is made up to 5 ml. with dehydrated alcohol and then made up to 50 ml. mark with chloroform.
4. Isopropylamine (IPA) reagent (30%, v/v): 15 ml. of IPA taken from a freshly opened ampule is diluted to 50 ml. with chloroform. This reagent should be prepared the day it is to be used.

5. Standard barbital (diethyl barbituric acid) solution: A chloroform solution of barbital is prepared containing 1 mg. per ml.

Procedure - Add 1 ml. of IPA reagent to 10 ml. of barbiturate chloroform solution in a cuvette and mix by twirling. One ml. of 1/25 M. CoCl_2 reagent is added. The solutions are mixed thoroughly and permitted to stand for 10 minutes before reading the percentage transmittance with the Coleman Junior Spectrophotometer at 560 m μ . A reagent blank consisting of 10 ml. of chloroform and 1 ml. each of the above reagents serves as a reference blank. A C-T curve is obtained by treating aliquots of the standard cobalt solution in exactly the same manner. The concentration of barbiturates in the unknown chloroform solution is obtained by extrapolation from the standard C-T curve (Figure 1).

Extraction of Barbiturates from Tissues and Body Fluids - Of the several methods suggested in the literature for the quantitative extraction of barbiturates from tissues and body fluids, that introduced by Levvy (34) appeared to be the most adaptable to this laboratory. The method can be described briefly as follows: Blood or macerated tissue is acidified with solid NaH_2PO_4 and mixed with anhydrous Na_2SO_4 . This is dried in a desiccator, pulverized, and packed into a Soxhlet thimble where it is continuously extracted with ether-petroleum ether (1:1). The extracts are decolorized with a charcoal-MgO mixture, filtered, evaporated to dryness, and the residue taken up with chloroform for the colorimetric determination. Experience indicated, however, that this method did not completely decolorize the extracts and failed to remove substances interfering with the final reaction. A number of modifications were therefore studied in an attempt to improve the procedure. While not completely satisfactory, it was found that more of the color and interfering materials were removed when the charcoal was mixed with the sample previous to continuous extraction than when the adsorbents were applied to the extracts. It was also found necessary to remove insoluble particles from the final chloroform solution by filtration. The colored cobalt-barbiturate complex is more soluble than the barbiturate itself, and by developing the color in the extraction flask it was found that less chloroform was required in transferring the sample, thus preserving a higher concentration of color in the final solution.

Procedure - Five grams of tissue or 5 ml. of body fluid is acidified with 2 grams of NaH_2PO_4 and mixed thoroughly with 10 grams of anhydrous Na_2SO_4 and 2 grams of activated charcoal. This mass is dried over CaCl_2 , pulverized, and transferred to a Soxhlet extraction thimble. A continuous extraction with a mixture of ether and petroleum ether (1:1) is carried on for 8 hours at 50°C. The solvent is evaporated at room temperature with the aid of a stream of air dried with CaCl_2 . Ten ml. of chloroform and 1 ml. each of 30% IPA and 1/25 M CoCl_2 reagents are then added to the dry residue, the resulting colored solution filtered, and the filter paper washed with successive portions of chloroform. The filtrate is made up to 17 ml. From this point, the colorimetric determination is carried out as described above.

By this procedure, crystalline residues were obtained from body fluid extracts which were almost entirely free of colored substances. However, the tissue extracts were still highly colored. Further modification of the method will be required before it can be applied routinely to tissue analysis.

Determination of the Purity of Tablets or Solutions - The Chemistry Department occasionally receives solutions of procaine where there is question of potency of solution used. The method of assay for procaine described in the U.S.P. (35) is clumsy and not too adaptable for small amounts of material. Since procaine possesses a primary aromatic amine, it was thought that the principle of diazotization followed by coupling to form a dye might be employed for its determination. The method used here is essentially that described by Bratton and Marshall for sulfonamides (36).

Principle: Procaine is diazotized with nitrous acid. The excess nitrous acid is destroyed with ammonium sulfamate and the diazo compound finally coupled with N-(1-naphthyl)-ethylene-diamine dihydrochloride. The intensity of the resulting purple-red color is measured using the spectrophotometer.

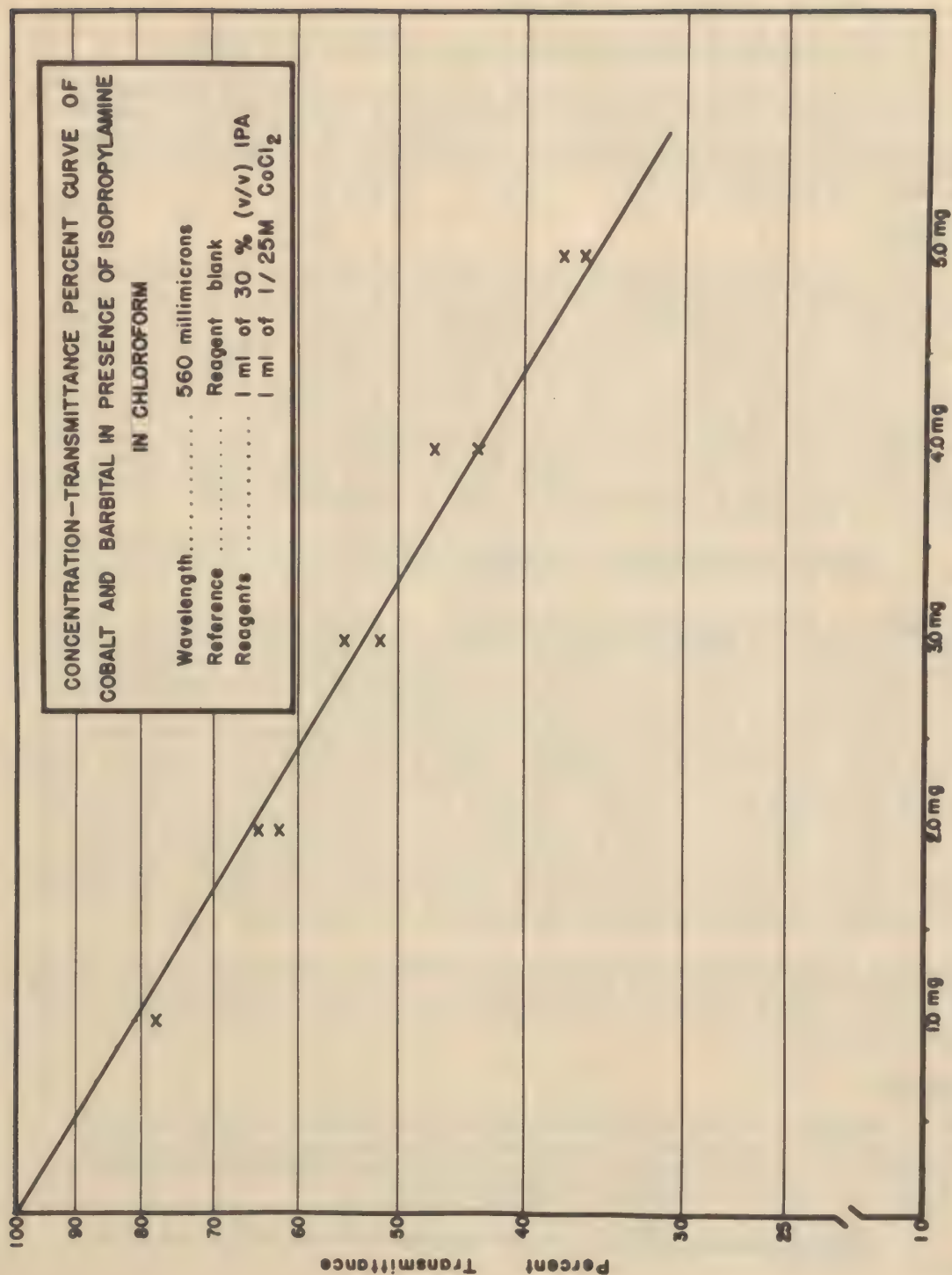


FIGURE 1

Reagents -

1. Hydrochloric acid, approximately 4 N.
2. Sodium nitrite, 0.1% solution.
3. Ammonium sulfamate, 0.5% solution.
4. N-(1-naphthyl)-ethylene-diamine dihydrochloride, 0.1% solution.
5. Procaine hydrochloride, standard solution containing 12.5 micrograms per ml. In the absence of pure procaine hydrochloride, it was extracted in crystalline form from tablets by taking advantage of its distribution between water and ether when converted back and forth between its salt and the free base. Final crystallization was made from hot ethanol.

Procedure -

1. Establishment of standard curve: Serial dilutions of the standard procaine solutions were made by pipetting 0, 0.25, 0.50, 1.0, 1.5, 2.0, 3.0, and 4.0 ml. volumes into separate curvette tubes and then adding sufficient water to make a total volume of 7.0 ml. Then 0.3 ml. of hydrochloric acid solution was added and the tubes shaken. One ml. of sodium nitrite solution was added and the tubes were shaken and allowed to stand for three minutes. One ml. of ammonium sulfamate solution was added and the tubes were shaken and allowed to stand for 2 minutes. One ml. of the N-(1-naphthyl)-ethylene-diamine dihydrochloride solution was added and the tubes were shaken and allowed to stand for 3 minutes before reading at 540 millimicrons with the Coleman Junior Spectrophotometer. The curve obtained (cf. below) is linear until a concentration of 25 micrograms of procaine hydrochloride per tube is reached (Fig. 2)

2. Assay of unknown sample of procaine: Appropriate dilutions of the unknown are made and these are treated exactly as described above for the standard solutions.

Determination of Pyribenzamine (triplennamine) - A search of the available literature yielded little that would aid this laboratory in the qualitative or quantitative estimation of pyribenzamine in body tissues. The chemical spot tests described by Haley (37) did not produce acceptable results, especially when amounts of pyribenzamine comparable to those expected to be found in tissues were employed. Perlman (38) has described a method for the quantitative determination of pyribenzamine and related antihistaminics which is based upon the cyanogen bromide reaction and the measurement of the resulting fluorescence. Since this laboratory is without a fluorophotometer, it was not possible to utilize this procedure. Dubost's (39) method for the determination of the antihistaminic compound, Antergan (N-dimethyl-aminoethyl-N-benzylaniline-HCl), whereby the nitroso derivative gives a green color with nitroso resorcinol (probably indaniline), was found inapplicable to the determination of pyribenzamine. Since pyribenzamine contains a pyridine rather than an aniline moiety, this failure was not surprising. Finally, recourse was made to the method of Dill and Glasko (40).

Principle - The colorimetric determination is based on a general procedure for organic bases described by Brodie and Udenfriend (41). It depends on the reaction of the organic base with methyl orange to form a colored complex salt which is soluble in certain organic solvents.

Reagents -

1. "Heptane" - Gasoline was distilled and the fraction between the temperature of 95 and 102 C. collected. This was shaken for five minutes with one-fifth volume of 10% NaOH. After separation, the hydrocarbon layer was washed successively with 1 N HCl and 3 separate portions of distilled water.

2. Ethylene dichloride (EDC) - A Japanese-manufactured EDC was purified as described for "Heptane".

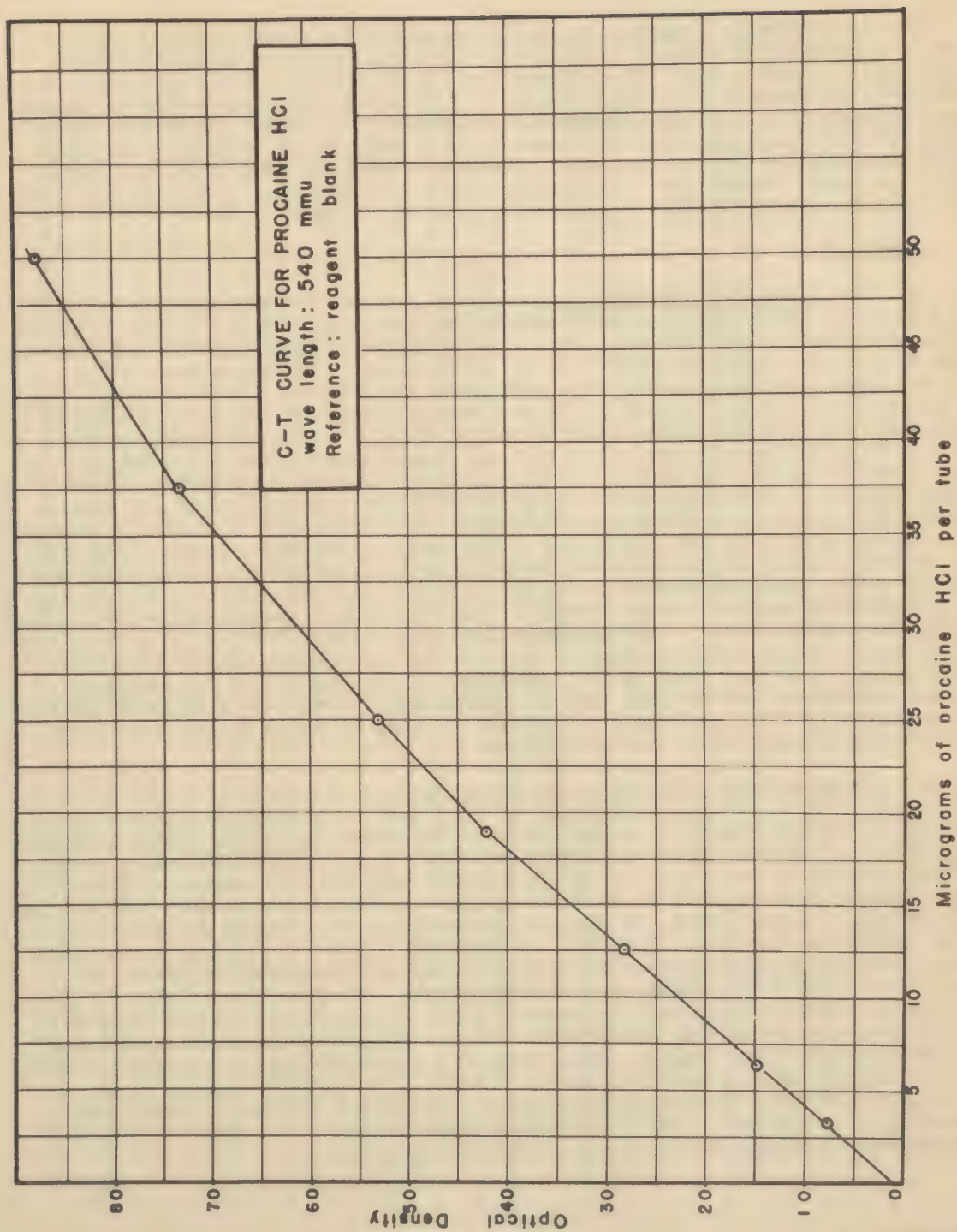


FIGURE 2

3. Methyl orange reagent - 0.5 M boric acid solution was saturated with methyl orange by shaking overnight in a mechanical shaker. Any undissolved methyl orange was filtered and the solution was washed with 3 separate portions of EDC.

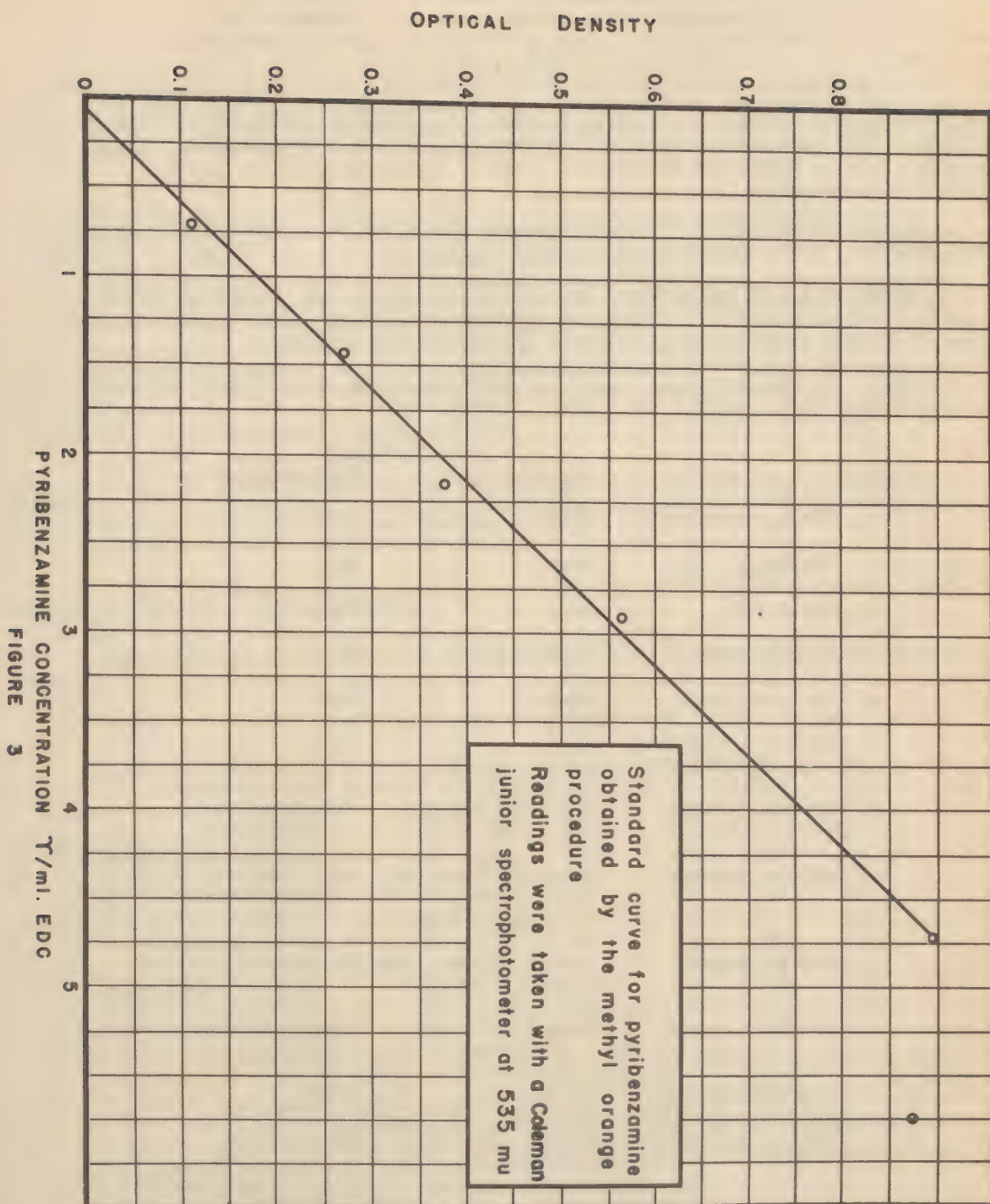
4. Acid-alcohol reagent - 2 ml. of concentrated sulfuric acid was mixed with 98 ml. of absolute ethyl alcohol.

5. Pyribenzamine Standard - Pure pyribenzamine being unavailable, it was necessary to isolate the compound from commercial tablets, which in preliminary tests were shown to contain about 25% pyribenzamine. Three 200 mg. tablets were pulverized and then shaken with absolute alcohol. The residue (the taste suggested lactose) was discarded. The alcoholic extract was evaporated to dryness. The residue was first extracted with ether and then taken up in a small amount of water. The pyribenzamine was extracted from the water with chloroform. The chloroform was removed by evaporation. Melting point of residue: 176 C. (theoretical: 189-192.5°C.). The 27.6 mg. of material obtained was dissolved in 25 ml. of water.

Procedure -

1. Establishment of standard curve - Two ml. of the standard solution was diluted to 24 ml. (88.2 micrograms pyribenzamine HCl per ml.). Two ml. of this solution, 2 ml. of 1 N NaOH, 5 ml. of water, and 20 ml. of EDC were shaken together for five minutes in a 50 ml. separatory funnel. After separation, the water was drawn off and the EDC layer pipetted into individual centrifuge tubes in the following amounts (ml.): 0, 0.625, 1.25, 1.875, 2.5, and 5. EDC was then pipetted into the tubes in amounts such that each tube contained a final volume of 7.0 ml. One ml. of methyl orange reagent was added to each tube, the tubes were stoppered (aluminum foil-covered stoppers), and the mixture was shaken for five minutes. As much of the methyl orange-water layer as possible was removed using an eye dropper, and the tubes were centrifuged. Five ml. from each tube was transferred to a colorimeter tube using a pipette. Care was taken to avoid addition of any of the methyl orange-water layer. Then 0.5 ml. of acid-alcohol reagent was added to each tube and readings were taken using the Coleman Junior Spectrophotometer set at 535 millimicrons. The tube containing 0 mcg of pyribenzamine was used as the reference tube. Thus, the tubes contained the following graded concentrations of pyribenzamine (mcg. per ml.): 0, 0.72, 1.43, 2.15, 2.87, 5.73. The resulting standard curve is given in the accompanying graph. This curve compares well with that given by Dill and Glazko (40) if one allows for variation between instruments and the fact that our pyribenzamine sample was not absolutely pure. Since benadryl HCl and pyribenzamine HCl have the same molecular weight, direct comparisons were thought to be justified (Figure 3).

2. Tissue extraction procedure and determination - Ten grams of tissue are homogenized with 10 ml. of water and 10 ml. of 0.1 N NaOH using a Waring blender. Fifteen ml. of the homogenate are transferred to a separatory funnel and shaken 15 minutes with 35 ml. of heptane. After the layers separate (it is sometimes necessary to centrifuge), 15 ml. of the heptane layer are pipetted into a 50 ml. separatory funnel. (The remaining heptane phase is processed through the EDC extraction step and used for spot testing). Six ml. of 0.1 N HCl is added and the mixture shaken for five minutes. After separation, the lower layer (EDC) is carefully allowed to run into a centrifuge tube. Then 0.5 ml. of methyl orange reagent (or more, if required) is added, the tube is stoppered with aluminum foil-covered stopper, and the mixture shaken for five minutes. As much of the methyl orange-water layer as possible is removed using an eye dropper, and the tube is centrifuged. Care is taken to avoid addition of any of the methyl orange-water reagent. After this, 0.5 ml. of acid-alcohol reagent is added and the reading taken as described above. A blank is prepared by shaking 10 ml. of EDC with 0.5 ml. of methyl orange reagent and 1 ml. of 1 N NaOH and proceeding as described for the sample at the stage of the determination. This blank is used as the reference tube when taking the spectrographic reading.



3. Recovery Studies - A sample of normal liver was homogenized and divided into 2 portions. One of these served as a liver blank, and to the other was added a known amount of pyribenzamine. Determinations were conducted on each as described above, with the following results:

Pyribenzamine added	35.32 mcg.
Pyribenzamine recovered	46.00 mcg.
Liver blank	9.90 mcg.

$46.0 - 9.9 = 36.1$

$36.1/35.32 \times 100 = 102\%$ recovery

The liver blank represents a "false pyribenzamine" content of about 6 mcg. per gram of liver. This particular sample of liver was autolyzed to a considerable degree. Two other livers, which were in a better state of preservation, gave blanks of 1.35 and 1.05 mcg. per gram.

A similar study employing stomach contents gave equally as good recovery of pyribenzamine. Stomach contents blanks were negligible.

4. Limitations of the method - It must be remembered that the method is not specific for pyribenzamine, but measures any organic base not common to body tissues or usual stomach contents that is soluble in the solvents employed.

Addenda - 1. The following reactions, differentiating benadryl and pyribenzamine, have been described by Haley (37):

<u>Reagent</u>	<u>Color Produced</u>	
	<u>Benadryl</u>	<u>Pyribenzamine</u>
a. HNO_3 , concentrated	None	None
b. $\text{BzH-H}_2\text{SO}_4$, 1:1	None	None
c. FeCl_3 , .9%	None	None
d. Diphenylamine	None	None
e. Furfural, H_2SO_4	None	None
f. Furfural, glacial acetic acid	None	None
g. Mandelin reagent	Red oily globules	Chocolate brown oily globules
h. Marquis reagent	Canary-yellow, red-dish, orange, then chocolate brown	Red, then deep reddish brown
i. Mecke's reagent	Canary-yellow, then reddish orange	Nut brown
j. Froehde's reagent	Canary-yellow to orange-red	Pale pink, then rust red
k. Concentrated H_2SO_4	Yellow	Brown
l. Resorcinol- H_2SO_4	Orange, then red-dish orange, and wine color on dilution	Yellow-green, then deep green, and olive-green on dilution

<u>Reagent</u>	<u>Color Produced</u>	
	<u>Benedryl</u>	<u>Pyribenzamine</u>
m. Furfural (1%) over H ₂ SO ₄	Orange-brown and yellow- green on shaking	Black, no change on shaking
n. Chromic acid	Orange-red precipitate	Orange precipitate
o. Foucey reagent	Cherry red	Cherry red

2. Properties of pyribenzamine (taken from N.N.R., 1949) - Pyribenzamine HCl occurs as a white crystalline powder possessing a bitter taste. It melts in the range 189-192.5 C. It is very soluble in water, soluble in ethyl alcohol and chloroform, and practically insoluble in benzene and ether. The pH of a 10% solution is from 6.4 to 6.6.

NUTRITIONAL RESEARCH: Effect of Thiamine Deficiency on Susceptibility of Mice to Japanese B Encephalitis - In 1949, Dr. White instigated a research program designed to determine whether there is a relationship between the nutritional state of an animal and its susceptibility to Japanese B encephalitis mouse brain virus. The preliminary results obtained during that year have been reported (41). Of the various nutritional deficiencies originally marked for study in the overall plan, thiamine avitaminosis was the first to receive attention. It was first necessary to determine the intake of thiamine that would just permit the animals to survive the experimental period without supplying optimal amounts of the vitamin. Repeated experiments showed this quantity to lie somewhere in the vicinity of 1 to 1.5 mg. of thiamine per kilogram of ration. The general plan of the experiment was as follows:

1. Mice were fed for a period of 2 weeks on diets containing known amounts of thiamine. The levels of thiamine intake were so adjusted that at least one group of mice received a suboptimal intake and another obtained much more of the vitamin than is known to satisfy the requirements of the animal.

2. Various dilutions of mouse brain virus were then injected intracranially with saline-injected mice used as controls.

3. The mortality of the mice was then followed until it appeared that no more deaths would occur (usually a period of about 11 days).

The results of several experiments have been combined in Table X below:

Table X. Effect of Thiamine Intake on the Susceptibility of Mice To Intracranially Injected Japanese B Encephalitis Virus.

<u>Mg. of thiamine/kg. of diet</u>	<u>Saline Control Mice</u>			<u>Virus-injected Mice</u>		
	<u>1.0</u>	<u>1.5</u>	<u>10.0</u>	<u>1.0</u>	<u>1.5</u>	<u>10.0</u>
A. No. of mice started	25	85	60	40	375	375
B. No. of mice injected (1)	17	66	56	33	356	346
C. No. of mice surviving in- jection (2)	15	64	45	29	325	319
D. Total survivors (3)	12	63	44	8	43	57
E. Percent survival (4)	80	98	98	28	13	18
F. Average survival time (5)	5.7	6.0	6.0	4.4	4.0	4.3

- (1) The number of mice surviving the preliminary 2-week feeding period.
- (2) The number of mice surviving 24 hours after the injection. It was considered that mice dying this early did so as a result of brain injury from the injection rather than from virus infection.
- (3) Mice surviving the entire experimental period.
- (4) D/C x 100
- (5) Average number of days survived by C minus D mice.

The difference between the survival of animals receiving the various intakes of the vitamin are not great enough, nor the sampling of mice large enough, to permit definite statements. However, from this data it would appear that the thiamine intake does not affect the mortality of mice experimentally infected with Japanese B encephalitis virus.

The problem was discontinued in July when, as a result of critical events in Korea, it becomes necessary to devote more time and laboratory space to more expedient problems.

DEPARTMENT OF PATHOLOGY

INTRODUCTION: As was true for all other Departments in the Laboratory, the work of the Pathology Department during 1950 was markedly affected by the outbreak of hostilities in Korea. It has been somewhat difficult to report this increased activity in an orderly and simple manner. For example, deaths due to battle injuries are listed under autopsies and discussed under battle injuries. Some are again discussed under the title of Lower Nephron Nephrosis or Japanese "B" Encephalitis. Similar overlapping was encountered with surgical specimens. The frostbite cases were received during December and studies are therefore incomplete. The availability, dependability, and accuracy of clinical records are mentioned in several places. These are intended as statements of fact and not of criticism since it is recognized that in time of intense medical activity there is little opportunity for doctors to record more than minimal clinical data.

SPECIMENS RECEIVED: During 1950, a total of 4,614 specimens were received in the Pathology Department as follows:

Human Autopsies	547
Surgical Specimens	3,681
Miscellaneous	386
Total	4,614

When compared with 1949, this represents a 27% increase in autopsies, a 15% increase in human surgicals, 79% decrease in miscellaneous specimens, mostly guinea pigs for tuberculosis, and a 16% decrease in total specimens examined. The decrease is merely statistical, however, the increased number of autopsies resulting in roughly three times the previous work.

Work records performed by the technicians were kept only during the last three months of the year as follows:

Paraffin blocks prepared	5,154
Blocks for frozen section	95
Routine H & E slides	10,076
Special stains	429
Fat stains	212
Total	15,966

AUTOPSIES: As of 1 January 1951, all but 30 of the 547 autopsies had been completed by the pathologists assigned to this department. Autopsy specimens fell into two large groups; those performed entirely by members of this Department, either in Tokyo or with the Advance Section, 406th Medical General Laboratory, at the 118th Station Hospital, Fukuoka, Japan, and those performed by officers assigned to the contributing hospitals. Table No. I lists the installations which performed these autopsies.

Tables No. II and III list the causes of death in these autopsies. It will be noted that 219 deaths were due to non-battle injuries of which 196 were due to accidents. Vehicular accidents, poisoning, drowning, and gunshot wounds made up the majority of these cases. As noted in past years, alcoholism in various degrees was an important primary or contributing factor in most of these cases. Medico-legal aspects of violent and unnatural deaths were a constant problem frequently requiring complete toxicological examination of tissues.

Of the 148 deaths not due to injury, arteriosclerotic heart disease, Japanese B Encephalitis, and polio contributed the largest number with 37, 38, and 10 instances respectively. Because of the policy of evacuating patients with chronic disease to the Z.I., tumors, tuberculosis, etc., were not frequently seen at autopsy. Several of the 10 tumor cases occurred in patients who were not Americans (Philippinos and Japanese war criminals). The 14 cases with cause of death undetermined include cases submitted with incomplete data.

Table I

Japan Logistical Command	424
406th Medical General Laboratory ..	240
Advance Section, 406th MGL	80
118th Station Hospital	20
(Prior to 1 September)	
Osaka Army Hospital	67
172nd Station Hospital	9
161st Station Hospital	4
35th Station Hospital	4
Far East Command other than JLC	79
Kycom Army Hospital	46
U. S. Philippine Scout Hospital ...	13
18th Medical Group	20
Korea (Army Hospitals)	40
8054th Evacuation Hospital	34
15th MASH	2
8217th MML	1
4th Field Hospital	1
171st Evacuation Hospital	1
7th Medical Battalion	1
Navy	2
Unknown	2

Battle injuries and Japanese "B" Encephalitis are discussed in separate sections. One case of typhoid and 1 case of smallpox originated in Korea.

The case of fatal rabies was described in a case report submitted as a joint publication by the Pathology and Virus Departments. In this case a full course of therapy was started on the ninth day after a dog bite and the patient died on the thirty-first day. The virus was isolated from the dog's brain and the patient's brain. Negri bodies were found in the dog's brain and in the brains of mice inoculated with tissue from both the dog and the patient, but not in sections of the patient's brain.

The case of blastomycosis occurred in a soldier who had been in Japan for one year. Autopsy showed widespread involvement including the skin, lungs, meninges, bone and prostate. The diagnosis was not made until autopsy at which time the soldier's organization was in Korea. No dependable information was available to support the possibility that the infection had occurred in Japan.

Table II. Summary of Causes of Death

Non-Battle Injuries	219
Accidental	196
Suicidal	19
Homicidal	4
*Disease	148
Infants	51
Cause Undetermined	14
*Battle Injuries	115

* Disease includes 10 cases with battle injuries also.

Table III. Cause of Death

Non-Battle Injuries 219

Accidental 196

Trauma, type not stated	9
Train	6
Vehicular	58
Fall	10
Airplane	21
Gunshot wound	22
Poisoning	29

*Ethyl alcohol	11
Methyl alcohol	1
Chloral hydrate and al- cohol	1
Pyribenzamine	1
Barbiturate	2
Carbon Monoxide	4
Alkaloids (morphine)	6
Carbon tetrachloride	1
Type undetermined	2

Electrocution	2
Anaesthetic	1
Crushing	1
Burns	8
Asphyxiation	3
Drowning	26
Freezing	1

Suicidal 19

Gunshot wound	18
Fall	1

Homicidal 4

Gunshot wounds	2
Stab wounds	2

Disease 148

Arteriosclerotic heart disease ..	37
Cerebral hemorrhage	6
Congestive heart failure.....	1
Atypical endocarditis with mural thrombosis	1
Acute bacterial endocarditis	1
Pericarditis	1
Pneumonia	3
Pulmonary tuberculosis	1
Tuberculous meningitis	1
Interstitial pneumonia	1
Blastomycosis, lung and meninges.	1
Laryngeal edema, acute	1
Laryngo-tracheitis, possibly diphtheritic	1
Ulcerative gastritis	1

Table III. Continued

Perforated peptic ulcer	1
Peritonitis, secondary to appendicitis ..	1
Typhoid	1
Bacillary dysentery	2
Mesenteric thrombosis	2
Infectious hepatitis	2
Toxic necrosis, liver	2
Cirrhosis	4
Post-hepatitis cirrhosis	1
Diabetes	2
**Lower nephron nephrosis	1
Glomerulonephritis	3
Meningitis, acute	3
Sub-dural hematoma	1
Sub-arachnoid hemorrhage	1
Rabies	1
*** Poliomyelitis	10
****Japanese "B" Encephalitis	38
Brain abscess	1
Exfoliative dermatitis	1
Smallpox	1
Post-abortion septicemia	1
Thrombocytopenic purpura	1
Leukemia	1
Transitional cell carcinoma, nasopharynx.	1
Bronchogenic carcinoma	2
Carcinoma, stomach	2
Carcinoma, caecum	1
Hepatoma	1
Carcinoma, prostate	1
Hemangioblastoma, brain	1
Infants	51
Stillborn	13
Premature	13
Congenital anomaly	10
Pneumonia	1
Asphyxia neonatorum	5
Acute gastroenteritis	1
Intussusception	1
Umbilical hemorrhage, secondary	1
Omphalitis	1
Pyelonephritis, acute	1
Hemorrhagic disease of newborn	1
Erythroblastosis foetalis	2
Traumatic	1
Cause Undetermined	14
****Battle Injuries	115
* Ethyl alcohol in significant amounts was found in many of the traumatic deaths but was not listed as the cause of death	
** 43 other cases are listed under battle injuries	
*** Includes 1 case with battle injury	
**** Includes 9 cases with battle injuries	
***** 10 additional battle injury cases are listed under medical; 9 under Japanese "B" encephalitis, and 1 under polio	

SURGICAL SPECIMENS: Malignancies - Among the 3,681 surgical specimens received, 140 showed the presence of cancer. These cases are shown in Table IV. Patients with skin tumors and other tumors easily accessible to therapy frequently were treated within this theater but other cases usually were returned to the Z.I. with a minimum of delay. The policy of giving the patient a duplicate histological section to hand carry to the installation in the Z.I. was followed in these cases.

The majority of the surgical specimens consisted of the usual routine type of material. However, it is of some interest to note that there was little difference in the number of these specimens submitted before and after 1 July, when the Korean conflict occupied so much attention. Thus in the first six months 457 appendices, 156 endometrial biopsies, 124 cervical biopsies, 41 pilonidal sinuses and 206 skin biopsies were examined as compared with 478 appendices, 129 endometrial biopsies, 119 cervical biopsies, 39 pilonidal sinuses and 195 skin biopsies for the last six months of 1950. The following table lists the more common routine surgical specimens examined during 1950:

Appendices	935
*Endometrial biopsies	285
Cervical biopsies	243
Skin biopsies	401
Nevi benign	105
Breast, male	43
Breast, female	93
Pilonidal sinuses	80
Thyroid	34
Gallbladder	40
Lymph Nodes	70

* Not including results
of therapeutic curettage show-
ing products of conception only

MISCELLANEOUS SPECIMENS: Most of these specimens were derived from animal sources and were submitted by various departments of the 406th Medical General Laboratory. Although 386 specimens were placed in this category, the actual number was larger since several specimens consisted of pools of tissues such as a group of about 20 skin biopsies of guinea pigs exposed to cercariae of Schistosoma japonicum. The studies for rabies (19 dog and 3 cat brains) showed a slight decrease from the 27 animal brains examined in 1949. However, 6 were positive for Negri bodies in 1950 and 5 in 1949.

As can be seen in Table V. guinea pigs for tuberculosis still make up the largest number of specimens examined in this group. However, there is a considerable variety of specimens.

BATTLE INJURIES AND DISEASE FROM KOREA: The specimens received by the Pathology Department related to injuries received in battle or to unusual exposure to disease in Korea, fall into two large groups: autopsy and surgical specimens. There was little overlapping or duplication of cases in the two groups. In several instances no surgical specimen was submitted due to operation having been performed in Korea. In very few instances is a patient represented by both autopsy and surgical material. The types and number of these specimens received through 31 December 1950, are listed in Table VI. All except the following are discussed in greater detail in separate sections.

The patient with smallpox had a typical severe infection. He denied any knowledge of having had a primary "take", and immunization record showed vaccination 28 July 1949, with "Immune" result, plus an attempt 1 July 1950, with no record of result. It is known that there was at least one other fatal case of smallpox in an American soldier in Korea, but no autopsy was performed. The patient with typhoid was a 21 year old white soldier with an overwhelming toxic infection. He showed no response to intensive chloromycetin treatment started on the sixth day of illness

Skin and mucocutaneous junctions:

Basal cell carcinoma, skin	35
Squamous cell carcinoma, skin, lip, etc.	20
Dermatofibrosarcoma, protuberans	1
Malignant melanoma	2

Breast:

Adenocarcinoma, breast	8
Intraductal papillary carcinoma, breast	2

Gastro-Intestinal System:

Carcinoma, stomach	1
Sarcoma, stomach	1
Adenocarcinoma, cecum	1
Adenocarcinoma, colon	2
Adenocarcinoma, rectum	3

Genito-Urinary System:

Transitional cell carcinoma, bladder	3
Papillary carcinoma, bladder	1
Seminoma, testis	2
Carcinoma in situ, cervix	4
Carcinoma, infiltrating, cervix	2
Adenocarcinoma, uterus	3
Pseudomucinous cystadenocarcinoma, ovary	1
Choriocarcinoma, uterus	2

Respiratory System:

Transitional carcinoma, nasopharynx	1
Squamous cell carcinoma, larynx	2
Bronchogenic carcinoma	1

Neurogenous:

Intracranial teratoma	1
Retinoblastoma	2
Retroperitoneal neuroblastoma	1

Lymphomas and Leukemia:

Hodgkin's disease	8
Giant follicular lymphoblastoma	1
Lymphoblastoma	1
Keticulum cell sarcoma	1
Lymphoblastic lymphosarcoma with leukemia	1
Leukemia	2

Mesodermal:

Neurogenic sarcoma	5
Neurofibrosarcoma	3
Fibrosarcoma	1
Granular cell myoblastoma (neurofibroma)	3
Osteochondrosarcoma	1
Osteogenic sarcoma	2
Multiple myeloma	1

Miscellaneous:

Mixed tumor parotid	5
Papillary cystadenocarcinoma, thyroid	1
Mestastic carcinoma, origin undetermined	2

Table V. Diagnoses in Miscellaneous Specimens

Animals for Tuberculosis	292
Positive	231
Negative	61
Animals for Rabies	26
*Positive	10
Negative	16
Animals for Smallpox	5
Animals for schistosoma (lots)	6
Animals for leptospira (lots)	4
Animals for Japanese "B" Encephalitis	10
Miscellaneous	43

* Including 4 mouse brains after intentional laboratory inoculation.

and died on the 13th day of illness. At autopsy, organisms were recovered from heart blood, lung, gallbladder, and intestine. Typhoid bacilli recovered from stools were sensitive to chloromycetin in vitro. He had the relatively infrequent complications of hemorrhagic lobar pneumonia, sub-arachnoid hemorrhage and myocarditis.

Tokyo Army Hospital has been designated the eye center for Japan and all of the eyes submitted came from this hospital. All were removed because of trauma. None were studied in this department, all being submitted to the Armed Forces Institute of Pathology.

The 28 specimens of central nervous system tissue were mainly portions of brain tissue removed at surgery either with a diagnosis of "cerebral fungus" or to determine the presence of suppuration.

The specimens of spleen, intestines, and arterio-venous fistulas showed nothing unusual.

Table VI. Autopsy and Surgical Specimens Resulting from Injury or Disease in Korea

Autopsies	157
Battle Injury	115
Battle Injury and JBE	9
Battle Injury and Polio	1
JBE	30
Smallpox	1
Typhoid	1
Surgicals	208
Extremities	70
Battle Injury	37
Frostbite and battle Injury	12
Frostbite	21
Eyes	89
Central Nervous System	28
Spleen	9
Intestine	6
Arterio Venous Fistula	6

AUTOPSIES ON WAR WOUNDS: From the outbreak of hostilities in Korea until 31 December 1950, the Pathology Department performed complete autopsies on or did microscopic examinations of tissues submitted by contributing laboratories in 125 deaths due to or associated with battle injuries, including 9 complicated with Japanese "B" Encephalitis and 1 with poliomyelitis. These represented a wide variety of injuries. Though these cases are only a small percentage of the deaths due to battle injury,

they do include, as far as we know, almost all of the cases of battle injury with subsequent death in an army hospital in Japan, plus a small representative group of those dying in hospitals in Korea. These cases, therefore, are a significant sampling of the causes of death where the battle injury is of such a degree as to permit evacuation through medical channels and hospitalization in rear echelons in Japan and, to a certain degree, in Korea.

When reading the following section, it is important to remember that information available in clinical records are frequently scanty or entirely lacking, due to the exigencies of the times; medical officers were too busy caring for the injured to write adequate notes, and the EMT tags were frequently lost. Further, several of these cases have not been completely studied as yet, and the results in these instances are, therefore based on gross examination only.

Missiles - Information about missiles is believed not to be completely reliable. There is some obvious overlapping of terms and probably a rather free use of the term "gunshot wound". It is probable that most of the 31 listed as shell fragments, mortar, and shrapnel, as well as some listed as gunshot wounds, were produced by mortar fire. Ten of the 31 patients had wounds of more than one region. Twenty-eight of the 58 patients with gunshot wounds had injuries of multiple regions. Table VII is obtained from available information.

Duration of Injury - The time between injury and death does not appear to be a significant or constant factor. Although there were some causes of death more than 5 weeks after injury, half of the patients died within a week of injury, and most within 2 weeks of injury. With certain notable exceptions, the injuries were considered grave and these patients could not tolerate transportation to the Z.I. Obviously, no information is available concerning patients who were evacuated to the Z.I. and subsequently died. Among the exceptions mentioned above were two cases with minor and moderate battle injuries who died of Japanese "B" Encephalitis, 3 cases of acute fat embolism, and a number of patients with slightly to moderately severe battle injuries who died of lower nephron nephrosis. Table No. VIII gives the available information.

Location of Injury - Table IX shows the regions of the body involved in war wounds in this group. It will be noted that injuries of more than one region occurred in 40 of the 125 patients. Data is not available to determine the regional distribution of non-fatal battle injuries received in Korea; however, it is known that there was a much larger percentage of extremity wounds than is shown in Table IX. The 35 with injury of the abdomen or abdomen plus some other region, who showed peritonitis at autopsy, was a prominent feature in this series. One factor may have been the difficulty encountered in evacuating patients over the poor Korean roads to the nearest medical facility where abdominal surgery could be performed. Undoubtedly, this resulted in delayed surgery. Information concerning the interval between injury and surgery is not available at the present time in these cases.

Anatomical Cause of Death - Table X gives the anatomical cause of death. Patients with wounds of more than one area are listed with the more seriously involved areas named first. However, it should be noted that only one of the 36 patients with head injury alone and 1 of the 8 patients with spine injury, developed lower nephron nephrosis.

Twenty-five of the 36 patients with head injury alone died as a direct result of that injury, and 7 others died of the head injury plus Japanese "B" encephalitis. Four died of pulmonary complications. Of the 8 with spinal injury alone, 6 died of pulmonary complication and 1 of myocarditis. The latter, a liberated POW, had a sacral wound which, in retrospect, was suspected of containing an unproven diphtheritic infection.

Only 4 patients had a wound of the thorax alone, while 12 had wounds involving both the thorax and abdomen. In 3 of the former group and 1 of the latter, intra-thoracic injury and complications were the cause of death. This suggested that thoracic injuries were either rapidly fatal or amenable to therapy. Abdominal wounds, on the other hand, more frequently resulted in death after an interval, because of peritonitis or peritonitis and lower nephron nephrosis.

Table VII. Missiles

Body Region	Unknown	GSW	Shell Fragments	Mortar	Shrap- nel	Gren- ade	Mine	Bay- onette	Crushing	Chemical Burns
Head	11	14	3	3	4	1				
Spine	1	4	1	1				1		
Thorax	1	1	1	1						
Abdomen	6	7	1	2		2			1	
Thorax and abdomen		10		1	1					
Extremities	2	6	2	1	1	1	3	1		1
Abdomen and extremities	1	5	2			1				
Thorax and spine		3								
Abdomen, Thorax, and Spine		1	1		1					
Head and Ex- tremities		1	2							
Abdomen and spine		2								
Miscellaneous	2	4		1	1					
Totals	24	58	13	10	8	5	3	2	1	1

Table VIII. Duration of Injury in Days

	Mean	Average	Extremes	Unknown
Head	10	12.6	1-52	7
Spine	11	19	2-65	
Thorax		4.5	2-7	2
Abdomen	13	14	2-33	2
Thorax and Abdomen	5	8	1-27	
Extremities	6	7.6	1-28	2
Abdomen and Extremities ...	3	5.2	2-12	
Thorax and Spine	1	1.3	1-2	
Abdomen, Thorax and spine..	8	12	1-27	
Abdomen and Spine	3	3	3	1
Miscellaneous	3	6.5	1-38	2
Cases with Lower Nephron ..	8	7.2	2-21	1
Nephrosis				

Table IX. Location of Injury By Regions

Head	36
Spine	8
Thorax	4
Abdomen	19
Thorax and Abdomen	12
Extremities	18
Abdomen and Extremities	9
Thorax and Spine	3
Abdomen, Thorax, and Spine	3
Head and Extremities	3
Abdomen and Spine	2
Miscellaneous..(Multiple)	8
Total	125

Table X. Anatomical Cause of Death and Regional Injury

Cause of Death	Head	Spine	Thorax	Abdomen	Thorax & Abdomen	Extremities	Abdomen & Extrem.	Thorax & Spine	Abdomen, Thorax & Spine	Head & Extrem.	Abdomen & Spine	Miscellaneous	Total
Brain Damage	14												14
Cerebellar Hemorrhage			1									1	2
Brain Abscess	5												5
Meningitis	5												5
Japanese "B" Encephalitis	7					1							8
JEB and Peritonitis							1						1
Polio						1							1
Pneumonia		4					1		1	1		1	8
Other Pulmonary	4	2	3					3					12
Myocarditis		1											1
Mediastinal Hemorrhage					1								1
Peritonitis				7	4		1		2		1	1	16
Fat Embolization						2				1			3
Shock							1						1
Anaesthetic						1							1
Lower Nephron Nephrosis	1			2	3	5	1						12
LNN with Peritonitis				10	3		4				1	1	19
LNN with Fat Embolism						3						1	4
LNN with Shock						2				1			3
LNN with Gas Infection						1							1
LNN with Meningitis		1											1
LNN Transverse Myelitis												1	1
LNN and Pulmonary					1							1	2
Tetanus						1							1
A.V. Fistula						1							1
Laryngeal Obstruction												1	1

It is known that more patients were evacuated to Japan with wounds of the extremities than for any other type of injury, and that the mortality rate was lower than for other injuries. Deaths in such cases were more frequently a result of a complication rather than a direct result of the injury. In addition to lower nephron nephrosis, which with various other conditions accounted for 11 of the 18 deaths, shock and fat embolization were important contributing factors. The patient who died of tetanus was a Turkish soldier who had a relatively minor wound of an upper extremity. The patient who died of the arterio-venous fistula had a rupture of the false aneurysm present and died of uncontrollable bleeding.

LOWER NEPHRON NEPHROSIS: Among the 125 deaths with war injuries, examination of available clinical records and of histological sections of the kidneys resulted in a diagnosis of lower nephron nephrosis in 43 or 34.4%. The following analysis of this group must be viewed with caution since records were incomplete. Only 2 transfusion reactions are noted in the records of the entire group of 43 patients. In other cases, there is only the statement that blood was given, but no description of the amount or type. It should be recalled that only Group "O" blood was sent to Korea and that this was given in some few instances without prior cross-matching. Also, this blood was marked "low" and "high" titer with the understanding that the former was to be used especially when the recipient's blood group was known to be "A", "B", or "AB". Rh type was also indicated on the label and Rh negative blood was intended for patients with known Rh negative blood type or who required multiple transfusions. When patients

were evacuated to Japan, blood, when given, was to be preceded by cross-matching, and group specific and Rh specific blood was to be given if at all possible. It should furthermore be realized that signs of transfusion reaction may frequently be missed due to anesthesia, coma, and limited laboratory facilities in the field.

Records, besides being unreliable concerning the use of and reaction to blood, also left much to be desired concerning the presence and degree of shock, and the severity of the battle injuries. It is recognized, of course, that under the circumstances the busy surgeons had neither time nor inclination to keep pretty records, and these explanations are intended only to emphasize that the following discussion is based on interpretations which may be inaccurate.

Table XI gives the incidence of lower nephron nephrosis in relation to regions and combined regions injured by war wounds. It is apparent that injuries of certain regions such as head alone were associated with a very low incidence of lower nephron nephrosis (2.7%) while other regions, particularly the abdomen (63%) and extremities (61%) had a high incidence of the condition. It is believed that several factors were involved in this regional association. Patients with head wounds were not usually given repeated or large blood transfusions. Further, only patients with less severe head wounds survived and could be evacuated to Japan. In such cases, shock was probably not a frequent or important complication. Destruction of tissue and chronic diffuse inflammation were likewise not important factors. However, patients with abdominal and extremity wounds frequently received numerous transfusions, were in shock for prolonged periods of time, and had large areas of suppuration and destruction of tissue.

Table XI. Incidence of Lower Nephron Nephrosis in Relation to Region of the Body Involved

	<u>No. of Cases</u>	<u>No. Cases Lower Nephron Nephrosis</u>
Head	36	1
Spine	8	1
Thorax	4	0
Abdomen	19	12
Thorax and Abdomen	12	7
Extremities	18	11
Abdomen and Extremities ...	9	4
Thorax and Spine	3	0
Abdomen, Thorax, and Spine.	3	1
Head and Extremities	3	1
Abdomen and Spine	2	1
Miscellaneous (Multiple) ..	8	4
Total	125	43

Table XII gives the incidence of lower nephron nephrosis according to regions involved with each region listed separately. Thus a patient with wounds of more than one region is listed under each region. Although this exaggerates the incidence of lower nephron nephrosis for head, spine and chest wounds, it still shows that lower nephron nephrosis was much more frequently found in wounds of the abdomen and of extremities.

Table XIII lists the severity of the wounds as determined from available information. Severe cases include all with a guarded prognosis and, therefore, all cases with brain injury, intestinal perforation, peritonitis, transverse myelitis, etc. It is, therefore, not surprising that most of the wounds were considered severe.

Table XIV lists the degree of shock present when the patient was first treated. Few of the records actually described the degree of shock present. This was estimated by the blood pressure, pulse rate, hematocrit, the amount of blood given, the type of injury, and the time interval between injury and treatment. When such deduction was not practical, the cases are listed as "not recorded".

Table XII. Incidence of Lower Nephron Nephrosis in Relation to the Individual Areas Involved

	<u>No. of Cases</u>	<u>No. of Cases Lower Nephron Nephrosis</u>	<u>Percentage of Cases with L.N.N.</u>
All Head Wounds	40	3	7.5
All Spine Wounds	20	5	25
All Chest Wounds	26	10	38.5
All Abdominal Wounds.	49	27	55
All Extremity Wounds.	37	20	54

Table XV lists the infections present at the time of autopsy in those patients who also had lower nephron nephrosis. It is evident that infection in more than one region was present in several patients, particularly those with abdominal and thoracic wounds. Although laparotomy had been performed in all of the cases of abdominal injury with intestinal perforation, all had peritonitis at autopsy. In 21 of these cases, the peritonitis was diffuse and in 7 it was localized either in the pelvis or upper abdomen. Seven of the 11 patients with pleuritis had chest wounds. In the remaining 4 patients, a diaphragmatic pleuritis resulted from extension of a diffuse peritonitis. Two of the 12 cases of cellulitis were instances of gas bacillus infection of amputated extremity stumps. In one case, Cl. perfringens, proven to be pathogenic by guinea pig inoculations, and Cl. putrificum, and Cl. sporogenes, both non-pathogenic for guinea pigs, were isolated at autopsy. In the other case, crepitus was present and subsequently disappeared after debridement and antibiotic therapy. The remaining 10 cases of cellulitis involved extremities (5 cases) and perianal and pelvic areas (5 cases).

Of the 5 cases of pericarditis, 3 were uremic in origin, one the result of direct extension of pleuritis, and the fifth was the result of pericardial perforation by a missile. In the last case, B. anitratum was cultured from the pericardial fluid.

Table XIII. Severity of War Wounds Complicated by Lower Nephron Nephrosis

Severe	32
Moderate	11

Table XIV. Degree of Shock in Patients who Subsequently Died with Lower Nephron Nephrosis

Severe	18
Moderate	9
Not Recorded ..	16

Table XV. Inflammation Present at Autopsy in Patients Dying of Battle Injury and Lower Nephron Nephrosis

Peritonitis	28
Pleuritis	11
Cellulitis	12
Pericarditis	5

Blood Transfusions - This is the one factor under greatest suspicion as the cause of lower nephron nephrosis. It is known that 41 of these 43 patients received at least one blood transfusion. In 6 cases, blood was given but the amount given is unknown, and in 2 cases there is no available record that a transfusion was given. As mentioned previously, only 2 records mention the occurrence of a transfusion reaction. In 1 case, "A" blood was given to a Type "O" patient through error. This patient received a total

of 1500 cc. of blood. The other patient received a total of 11,500 cc. (23 pints) of Type "O" blood because of massive hemorrhage from an iliac artery.

Table XVI lists the amount of blood given these patients. The extremes were 1 and 52 pints. The mean was 3 pints and the average for all patients was 6.2 pints. The patient who received 52 pints of blood had massive bleeding from gastro-epiploic vessels following gastric resection.

Table XVI. Amount of Blood Given Patients Who Subsequently Died of Battle Injury and Lower Nephron Nephrosis

<u>Pints of Blood</u>	<u>No. of Patients</u>
1	7
2	5
3	8
4	4
5	2
6	1
7	1
8	2
9	1
11	1
13	1
23	1
52	1
Unknown	8
Amount unknown	6
No record	2

It was difficult in some cases to estimate whether the lower nephron nephrosis present was an important factor in producing the fatal result or whether it was an incidental finding unrecognized clinically and discovered only by histologic study of the kidneys. Undoubtedly, some of the patients had injuries so severe that the prognosis was very poor, regardless of the presence of lower nephron nephrosis. Other patients might have survived but for the added insult of the kidney lesion. Still another group suffered relatively moderate wounds and, having received adequate medical care to combat shock, blood loss and infection appeared in fairly good condition with a favorable prognosis. Death in the last group was directly attributed to lower nephron nephrosis. Table XVII, which lists the available clinical causes of death, indicates how infrequently lower nephron nephrosis was considered important in the fatal outcome. When this is compared with Table XVIII which lists the number of patients with clinical and laboratory findings supporting the diagnosis of lower nephron nephrosis, it would appear that the clinicians either did not clearly appreciate the importance of this complication or preferred not to include this diagnosis among causes of death in patients with battle injuries.

Table XVII. Frequency of Lower Nephron Nephrosis as a Clinical Diagnosis of Cause of Death in 43 Patients Dying of Battle Injury and Lower Nephron Nephrosis

<u>Diagnosis</u>	<u>No. Patients</u>
Lower Nephron Nephrosis	12
Possible Lower Nephron Nephrosis	1
Uremia	3
Hemolytic transfusion reaction with uremia	1
Total	17

Table XVIII. Frequency of Clinical Evidence of Presence of Nephrosis in Patients Dying of Battle Injury and Lower Nephron Nephrosis

<u>Evidence</u>	<u>No. Patients</u>
Oliguria	4
Oliguria and Azotemia	12
Oliguria and Hypertension	1
Uremia	12
Oliguria, azotemia, and hypertension	1
	—
Total	30

Table XIX is an attempt to evaluate the severity of the lower nephron nephrosis and the role it played in the death of these patients. The criteria for this evaluation are based on both clinical and autopsy findings. The criteria for the microscopic estimation of the severity and duration of the lesion of lower nephron nephrosis are still in the formative stage, and include the number of pigmented casts, the amount of interstitial edema, and the presence of peritubular foci of inflammation. It is planned to do special stains for iron pigment as part of a further study to determine the etiology of the lower nephron nephrosis (1).

Table XIX. Estimated Severity of L.N.N. Based on Clinical and Autopsy Findings in Patients Dying of Battle Injuries and L.N.N.

<u>Clinical Diagnosis</u>	<u>Degree of LNN (Clinical & Autopsy Estimation)</u>			
	<u>Severe</u>	<u>Moderate</u>	<u>Mild</u>	<u>Total</u>
L.N.N.	6	6	0	12
Possible L.N.N.	1	0	0	1
Uremia	2	0	1	3
Transfusion Reaction	0	1	0	1
Not diagnosed	3	11	12	26
	—	—	—	—
Total	12	18	13	43

Autopsy Findings - The microscopic lesions of lower nephron nephrosis have been described in the literature (2). All of the cases in this group met the requirements for this diagnosis. However, it is of interest to list, as in Table No. XX, the total weights of both kidneys in these cases. Combined weights ranged from 225 grams to 530 grams. In one case, a nephrectomy had been performed and the remaining kidney weighed 225 grams. The average weight was 436 grams and the mean 450 grams.

Table XX. Weight of Kidneys in Fatal Cases of Battle Injury and Lower Nephron Nephrosis

<u>Combined Kidney Weight (grams)</u>	<u>No. Patients</u>
200 - 300	3
300 - 400	10
400 - 500	16
500 - 600	12
Unknown	2
Extremes 225 - 530	

Table XXI lists the intervals, in days, between injury and death in these cases. Although the range is from 2 to 31 days, the average was 9.9 days, the mean 8 days, and 35 cases (80%) died within 10 days after initial injury. This suggests that the shock and trauma at the time of injury or the immediate treatment for the injury, in most

cases, were the important factors in producing the lower nephron nephrosis, since lower nephron nephrosis terminates in death in 75% within 8 days from onset (2). The cases with longer interval from injury to death had the onset of signs and symptoms at some unknown interval after the time of injury.

Table XXI. Interval Between Injury and Death

<u>Days</u>	<u>No. Patients</u>	<u>Days</u>	<u>No. Patients</u>
2	2	11	1
3	3	12	1
4	2	13	1
5	2	19	1
6	5	20	2
7	4	21	1
8	4	27	2
9	6	31	1
10	5	Average - 9.9 days, mean - 8 days	

Summary - In 125 autopsies in cases of death following war wounds some degree of lower nephron nephrosis was found in 43 instances (34%). In many instances the condition was unrecognized clinically. A preliminary discussion is given of possible etiology, related factors, and severity of the condition. The relative importance of shock and transfusion reaction is undecided. There appears to be a definite association of this renal damage with abdominal wounds, especially in the presence of peritonitis, and in wounds of the extremities.

AMPUTATIONS IN BATTLE CASUALTIES: During 1950 a total of 75 amputated extremities were received by the Pathology Department. Only 3 of these were received prior to 1 July, and only 5 of the 75 were non-battle injury. These 5 are listed below and are then disregarded in all further discussion:

Non-Battle Injuries

Accidental flare explosion	1
Gunshot wound	1
Extensive burns (from plane crash)	1
Trauma by railroad train	1
Crushing injury by machine	1

Total	<u>5</u>
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Source of Specimens: Because of problems of shipping and fixation of specimens, it was not customary for hospitals outside of the Tokyo Area to send amputated extremities to the 406th Medical General Laboratory. With the onset of cold weather, Osaka Army Hospital was designated as the frostbite center for the Far East Command. An officer was assigned from the 406th Medical General Laboratory to this hospital to serve as Laboratory Officer, and plans were made for a thorough study of any specimens which might become available. Tokyo Army Hospital and Osaka Army Hospital were then the two sources submitting amputated extremities. All 37 of the primarily traumatic specimens, and 11 of the frostbite specimens came from Tokyo Army Hospital. Twenty-two of the frostbite specimens came from Osaka Army Hospital.

Types of Battle Injury - Table XXII shows the distribution of these submitted specimens with relation to the type of injury.

Table XXII

	<u>No. Extremities</u>	<u>No. Patients</u>
War Wounds	37	37
Missile wounds	32	
Land mine trauma	2	
Agents unspecified ...	3	
Frostbite plus war wound	12	8
Frostbite alone	21	11
	—	—
Total	70	56

As can be seen in Table XXII, all amputations because of battle injury alone resulted in single amputations, while frostbite tended to require multiple amputation. The latter will be discussed subsequently.

War Wounds - 37 Cases - The necessity for amputation was usually obvious in the specimen. There was severe trauma with extensive bone and soft tissue damage, or there was damage to major vessels with ischemic infarction and necroses, or there was a combination of these injuries. Frequently associated with these injuries, there was extensive bacterial infection and sometimes evident accumulation of gas in the tissues.

Amputations apparently were performed with the object of preserving the longest possible stump so that operation through a fracture site or through areas of extensive soft tissue damage was not uncommon. In some instances, it is known that fractures were present proximal to the level of amputation. Table No. XXIII shows the incidence of the various types of major injuries and Table XXIV shows the sites of these types of trauma. Table XXV shows the level of amputation. It is evident that there were many more lower than upper extremity amputations and that interference with circulation was usually an important factor in these cases. This was true in more than half the fracture cases, all of which were of the compound comminuted type.

The large number of cases with vascular obstruction (Table XXVI) indicates the importance of this factor as an indication for amputation. Such extremities showed extensive ischemic necrosis and were frequently infected. There was suppuration, foul odor, one or two cases contained maggots, and in 3 instances the picture was of such a character that a clinical diagnosis of gas gangrene was made.

Bacteriologic Studies - In 12 specimens there was ischemic infarction without evidence of significant bacterial infection grossly. In 10 of these vascular occlusion by surgical ligation or by thrombosis was demonstrated. Bacteriologic studies were not performed on these 12 cases, but were performed on the remaining 25 extremities. These studies were carried out as a cooperative effort with the Bacteriology Department. Their findings and investigations will be presented in greater detail in a separate report.

Bacteriological examinations were directed primarily at determining the presence of anaerobes, particularly the Clostridia. In a few instances, when time and work permitted, the aerobic flora was also investigated. Since many of the specimens were received in December, these investigations are still incomplete.

Depending on the extremity and using as sterile a technique as possible, pieces of deep tissue from many sites were excised and identified as to location. These specimens were taken from traumatized and non-traumatized, gangrenous and non-gangrenous portions of the limbs, as well as from areas with good and poor vascular supply. A portion of each piece of tissue was set aside for histological examination and the remainder submitted for culture including, if possible, a titration for level of any antibiotics present. In most cases, only the pilot studies have been reported. These were usually a portion of tissue from the most suspicious area, cultured for the presence of Clostridia.

Table XXIII. Types of Major Injuries

Fractures, compound, comminuted	26
with vascular occlusion	15
with massive trauma	8
without vascular occlusion or massive trauma ...	3
Vascular occlusion without fracture	11
Total	37

Table XXIV. Sites of Battle Injuries

Fractures	26
Femur	8
Tibia and Fibula	9
Tibia	3
Femur and Fibula	1
Tarsals and Metatarsals	3
Humerus	1
Phalanges	1
Vascular occlusions (including 15 with fractures)	26
Femoral	6
Popliteal	10
Tibials	3
Plantar	1
Brachial	2
Ulnar and Radial	1
Uncertain*.....	3

* Uncertain: Cases of obvious vascular occlusion with site or type of obstruction not demonstrated in specimen and clinical data not available.

Table XXV. Level of Amputation

Upper Extremity	5
Middle 1/3 of arm	2
Upper 1/3 forearm	2
Metacarpo-phalangeal	1
Lower Extremity	32
Disarticulation, hip	1
Middle 1/3 thigh	3
Distal 1/3 thigh	13
Proximal 1/3 leg	3
Middle 1/3 leg	8
Distal 1/3 leg	3
Transtarsal	1

Table XXVI. Types of Vascular Occlusion

Traumatic	13
Thrombosis **	9
Severence or laceration	4
Surgical ligation	10
Uncertain*	3

* Uncertain: Cases of obvious vascular occlusion with site or type of obstruction not demonstrated in specimen and clinical data not available.

** Thrombosis: Adjacent bone and soft tissue damage present but without demonstrated severence or laceration of the vessel.

Table XXVII gives the present status of these studies. It should be noted that all of the incompletely studied cases are presumably positive for Cl. perfringens on the basis of acrolein tests, a test which the Bacteriology Department has found generally reliable.

Table XXVII. Bacteriologic Study of Amputated Extremities

Extremities cultured for <u>Clostridia</u>	25
Anaerobic culture not complete	6
Anaerobic culture completed	19
<u>Clostridia</u> recovered	16
<u>Cl. perfringens</u> alone	6
<u>Cl. perfringens</u> plus other	3
<u>Clostridia</u>	
<u>Clostridia</u> other than <u>Cl. per-</u>	7
<u>fringens</u>	
Negative for <u>Clostridia</u>	3

Since biochemical and pathogenicity tests have not been completed in 6 cases, only the 19 completed cases are considered in the identification tables below. In these cases, the strains of Cl. perfringens, Cl. septicum, and Cl. sordelli were proven pathogenic by guinea pig inoculation. The single instance of isolation of Cl. tetani was from a patient who showed no clinical evidence of tetanus although the organism was capable of producing toxin as shown by animal inoculation. The other Clostridia isolated were non-pathogenic to guinea pigs. Tables XXVIII and XXIX give the results of these cultures.

Table XXVIII. Species Isolated

<u>Cl. perfringens</u>	9 extremities
<u>Cl. putrificum</u>	5 extremities
<u>Cl. bifermentans</u>	4 extremities
<u>Cl. cochliorium</u>	2 extremities
<u>Cl. sporogenes</u>	2 extremities
<u>Cl. sordelli</u>	1 extremity
<u>Cl. septicum</u>	1 extremity
<u>Cl. tetani</u>	1 extremity
<u>Cl. tertium</u>	1 extremity
<u>Cl. sp. unidentified</u>	1 extremity

Table XXIX

Extremities with 1 <u>Clostridium</u> species ...	11
Extremities with 2 <u>Clostridium</u> species ...	2
Extremities with 3 <u>Clostridium</u> species ...	1
Extremities with 4 <u>Clostridium</u> species ...	1
Extremities with 5 <u>Clostridium</u> species ...	1

Early in the study, it was apparent that the recovery of pathogenic Clostridia in amputated extremities was not synonymous with clinical gas gangrene. In several instances, it was a distinct surprise to learn that these organisms had been recovered.

In the following discussion, the 6 cases still incomplete but presumably infected with Cl. perfringens (positive acrolein test) are included. In 9 cases Cl. perfringens was recovered from soft tissue wounds of extremities infected as a result of vascular occlusion. In none was there clinical evidence of gas gangrene.

Gas bubbles or crepitus was found in 11 extremities. In 6 of these, Cl. perfringens was proven to be present, and in the remaining 5 cases, other Clostridia were present.

Table XXX shows the relation between Clostridia and presence of gas bubbles and/or crepitation in the extremity on dissection.

Table XXX. Relation Between Clostridia and Local Evidence of Gas Infection

	<u>Gas Present</u>		<u>Gas Absent</u>
<u>Clostridia</u> absent	3	0	3
<u>Cl. perfringens</u> present	15	6	9
<u>Clostridia</u> other than..	7	5	2
<u>Cl. perfringens</u>	—	—	—
Totals	25	11	14

It is difficult to assess the role played by antibiotics in these clostridial infections. Practically every patient received intensive antibiotic therapy, and in the opinion of the clinicians, with considerable benefit in limiting extension of infection. However, on examining the amputated extremities, other factors appeared at least as important. These were a selected group of patients in that their injuries were of such a degree as to permit evacuation from Korea to Tokyo and delayed amputation. Gangrene and necrosis, as indicated above, were usually related to vascular occlusion and massive trauma rather than infection. Little of the antibiotics, it is suspected, would enter infarcted areas and in contrast little or no toxic products would enter the body circulation. Clinically, these cases would not fall into the classical description of gas gangrene, i.e., a severe rapidly spreading very toxic infection progressing along tissue planes and associated with gas formation. The general impression was that these were anaerobic organisms growing in a previously prepared environment of necrotic tissue and showing only a moderate tendency toward extension. Such infections are described in the literature as anaerobic cellulitis (3).

It is obvious that the presence of Clostridia in necrotic tissue is not the only requirement for the diagnosis of gas gangrene. There must also be evidence that the organisms are capable of spreading beyond the previously prepared ischemic necrotic tissue. No autopsies of death due to gas gangrene were received by the Pathology Department.

EXTREMITIES AMPUTATED FOR FROSTBITE: Prior to 31 December 1950, a total of 33 frostbitten amputated extremities or portions of extremities from 19 patients were received in the Pathology Department. Nine additional frostbitten extremities amputated from 5 patients during December were received from Osaka Army Hospital in the first week of January 1951. This last group is included in the following discussion, although they are not considered in the totals reported for the year 1950 (Table XXII).

All but two of these specimens were lower extremities. It is known that, in 3 instances, amputation of the opposite lower extremity had been previously performed, although the specimens were not submitted to this laboratory. In addition, 1 patient who had a single lower extremity amputated had severe frostbite of the opposite lower extremity and subsequent amputation was anticipated. Thus, of 24 patients whose frostbite was of such a severity as to require amputation, only 4 escaped with loss of a single extremity. Table XXXI details this information. Again, it must be pointed out that this is a selected group of patients whose frostbite and associated injuries were of such a nature as to permit evacuation to Japan but also required amputation.

Table XXXI

	<u>Patients</u>	<u>Specimens</u>
Quadruple amputations	1	4
Double lower extremity amputations ..	15	30
Single lower extremity amputations*	8	8
Total	24	42

* 3 of these patients are known to have had amputations of the opposite lower extremity, and 1 of these patients is expected to lose the opposite lower extremity. Since these specimens have not been received by this laboratory, they are listed here as single lower extremity amputations.

In most cases, amputation was delayed until a line of demarcation was evident. In some cases with both frostbite and battle wounds of the extremity, early amputation was required. Although the indication for amputation was the battle wound, these cases are included in this group because frostbite was evident. When amputation was delayed, it was generally above the line of demarcation, in order to provide a practical and serviceable stump. Tables XXXII through XXXV show these relationships.

Frostbite was complicated by battle injury in 13 patients who lost 21 extremities. To a certain extent, this required a higher level of amputation. Fifteen of these 21 extremities were the sites of battle injury. Unquestionably, the presence of battle injury had a deleterious effect upon the course of the frostbite. However, it is impossible to judge, at this stage of the investigation, how important this effect was. The following case illustrates the problem: A soldier with bilateral frostbite of the feet and with gunshot wound of one foot required amputation only of the injured foot. In this case it was felt that the battle injury would not of itself have required amputation, but with frostbite the amputation was necessary. According to histories obtained, many of these patients were exposed to prolonged cold and immobilization after being wounded. Tables XXXVI and XXXVII show the type of wound and the number of extremities involved.

It is of interest that 9 extremities were from 4 patients who had been captured and released by enemy forces. Three of these men had been wounded and captured. Their shoes were taken from them and they lay in the snow, exposed to the cold for a considerable period of time. Quadruple amputation was required in 1 case, bilateral amputation in 2 cases, and a single amputation in the fourth case. Such information was not ordinarily available. Frequently, the surgical Form 515 stated only "WIA in Korea" or "Frostbite, Korea". In December, it became possible to assign the study of these extremities to one pathologist. He reviewed the clinical records of patients scheduled for amputation at Tokyo Army Hospital, made rounds with the surgeons, interviewed the patients, and witnessed the operation. A pathologist at Osaka Army Hospital attempted to follow a similar procedure. This proved to be too time-consuming. It is now planned that simple post-operative interview will be made, should additional amputated extremities be submitted for examination. It is planned, time, personnel and space permitting, to study the microscopic changes in some detail. It is hoped that it will be possible to study by special stains changes in connective tissue, vessels and nerves, and to make a search for arteriovenous anastomoses of the cutaneous glomus type. For this reason, the entire amputated specimen is being retained in formalin at this laboratory. To permit future orientation, the level of amputation, the line of demarcation, and the origin of each tissue block is indicated on outline drawings of the extremity at the time the specimens are examined.

The relationship between frostbite and war injury still requires considerable study. It is hoped that the available material will permit such a study.

JAPANESE B ENCEPHALITIS: It had been anticipated that fatal cases of Japanese B encephalitis would come to autopsy during the summer of 1950. Plans were therefore made, during the preceding spring, for a coordinated study including epidemiologic, virus, and pathological investigation. The following preliminary report includes work from these other groups as they affect the pathologic findings.

The original plan set up certain procedures to be followed. These included: (1) Autopsy to be performed as soon after death as possible; (2) the head was to be opened first and specimens for virus study were to be removed from each cerebral hemisphere with sterile precautions; (3) tissue for virus study was to be rapidly frozen and kept cold until inoculated into mice, or placed in buffered glycerin for transmission to the virus laboratory; (4) the entire remaining brain, and when possible spinal cord, was to be fixed in formalin for complete histologic study. The outbreak of the Korean conflict disrupted these plans to a certain extent. The tactical situation did not permit the detailed procedures planned and, as a result, only a limited autopsy was performed in most of the known deaths occurring in Korea from Japanese B encephalitis. Fortunately, a Medical Officer who was familiar with these plans had been transferred from this Department as Laboratory Officer to the 8054th Evacuation Hospital. Despite many difficulties the brain was examined in each fatal suspect case and material for virus isolation and for histological study was obtained. Other pathologists from the 406th

Table XXXIII. Interval Between Injury or Onset of Symptoms and Amputation

<u>Interval</u>	<u>No. Patients</u>
2 days*	1
12 days	1
14-20 days	14
21-27 days	3
33 days	1
Unknown	4

* Complicated by compound commuted fracture of tibia and fibula.

Table XXXVIII. Level of Demarcation

	<u>No. Specimens</u>
Middle 1/3 of leg	2
Lower 1/3 of leg	6
Maleoli	10
Tarsals	5
Mid-metatarsal	3
Distal metatarsal	5
Metatarsal-phalangeal	9
Wrists	2
Total	<u>42</u>

Table XXXIV. Level of Amputation

	<u>No. Specimens</u>
Lower 1/3 thigh*	1
Upper 1/3 of leg	2
Lower 1/3 of leg	24
Distal metatarsal	5
Metatarsal-phalangeal	8
Lower 1/3 of forearm	2
Total	<u>42</u>

* Complicated by GSW of thigh.

Table XXXV. Relation of Amputation Site to Demarcation Level

Above demarcation level	36
At demarcation level	6

Table XXXVI. Type of Wound

Missile wound	10 patients
Missile wound plus clostridial infection ..	2 patients
Bayonette wounds	1 patient

Table XXXVII. Extremities Involved by War Wound

	<u>Patients</u>	<u>Extremities Wounded</u>	<u>Extremities Amputated</u>
One of 2 or more amputated extremities*	4	4	10
Involving the single amputated extremity	7	7	7
Involving both of 2 amputated extremities	2	4	4

* One patient with single extremity wound had quadruple amputations, 3 with single extremity wound had double lower extremity amputation.

Medical General Laboratory were assigned to various hospitals in Japan, and were able to do more thorough autopsies. From most of these, the entire brain was preserved.

The original plan included an intensive study of the histologic lesions of Japanese B encephalitis, including the study of specially stained sections. Because of the pressure of work and the limitations of personnel and space, this portion of the study is incomplete at the present time. However, routine H & E survey sections from the major portions of the brain in all the cases submitted have been examined.

A total of 39 autopsies were submitted to or performed by the members of this Department in 1950. Five are believed to have originated in Japan and 34 in Korea. Since some, though not all of the soldiers passed through Japan on their way to Korea, it is possible that infection occurred in Japan. Although it is not known in how many instances this could have occurred, it is believed that it applied to only a few, if any, cases. Because symptoms in all of these cases first appeared in Korea, they are listed as cases originating in Korea. From the available statistics, the case fatality rate for Japan was therefore 19%, and for Korea 10.9% (26 cases reported in Japan and 311 cases in Korea). Table XXXVII lists the medical installations where death occurred.

Table XXXVII. Hospitals Where Death Occurred

	<u>No. Cases</u>
<u>Japan:</u>	
Tokyo Army Hospital	6
361st Station Hospital	2
Yokosuka Navy Hospital	1
Osaka Army Hospital	4
118th Station Hospital	6

Korea:

8054th Evacuation Hospital ...	19
USS Consolation	1
(See Department of Virus and Rickettsial Diseases)	

Epidemiology - There were 5 fatal cases of Japanese B Encephalitis originating in Japan and 34 originating in Korea. Although onset of symptoms occurred approximately two weeks earlier in the fatal cases originating in Japan, two cases appeared in Korea during the time fatal cases were having their onset in Japan. All of the deaths in Japan from Japanese B encephalitis occurred before there were any deaths in Korea. Table XXXVIII shows the time of onset and time of death by weeks. It is apparent that the outbreaks in both Japan and Korea were rather sharply limited and the deaths were similarly closely grouped chronologically.

Table XXXVIII. Time of Onset and Death in Cases of Fatal JBE by Weeks

<u>Date</u>	<u>Onset in Japan</u>	<u>Death</u>	<u>Onset in Korea*</u>	<u>Death</u>
Aug. 6-12	1	0	0	0
13-19	3	2	1	0
20-26	1	3	1	0
Sept. 27- 2	0	0	5	6
3- 9	0	0	4	2
10-16	0	0	15	7
17-23	0	0	3	11
24-30	0	0	3	6
Oct. 1- 7	0	0	0	1
Dec. 8-	0	0	0	1
Totals	5	5	32*	34

* Onset unknown in 2 cases

Age - In 9 cases the age was unknown. In the remaining 30 cases, it appears that the age distribution reflected the age distribution of the exposed military population. However, no conclusion is warranted because of the small number of cases involved. Table XXXIX gives this data.

Race and Sex - Of the 39 patients, 34 were white male American soldiers, 2 colored American soldiers, and 1 a white British soldier. Two of the five cases occurring in Japan were in females, one being a Russian and the other an American. Table XL gives the data.

Presenting Symptoms - The majority of patients with Japanese B encephalitis in Korea were not immediately diagnosed. Table XLI lists the available initial diagnoses as made at the first medical installation.

Table XXXIX. Age Distribution of Fatal Cases of Japanese B Encephalitis

<u>Age (years)</u>	<u>Number</u>	
17	3	
18	3	
19	5	
20	4	
21	6	
22	2	Average: 21.9 years
23	1	Mean: 21 years
25	3	
29	1	
37	1	
43	1	
Unknown	9	
Total	39	

Table XL. Race and Sex

American males (white)	34
American males (colored)	2
American female (white)	1
British male (white)	1
Russian female (white)	1
Total	39

Table XLI. Initial Diagnoses in Fatal Cases of Japanese B Encephalitis

FUO	9
FUO with convulsions	1
FUO with hysteria	1
Battle fatigue	1
"Neuropsychiatric"	2
"Neuropsychiatric" and malaria	1
Malaria	2
Dengue fever	1
Encephalitis	3
Total	21

Immunization - Reliable and complete data on immunization for Japanese "B" encephalitis is not available at the present writing. Therefore, no comparison can be made of the course of the disease, severity and types of histologic changes in immunized and non-immunized patients.

Duration of Fatal Cases of JBE - Duration of disease is shown in Table XLII. Omitting the one prolonged case, the average duration from recorded onset of symptoms to death was 5.1 days and the mean was 5 days. The extremes were 1 day and 13 days.

Diagnosis - In the fully proven cases, diagnoses were made clinically, histologically, and by virus isolation. In all but 6 cases, which will be described later, a clinical diagnosis was made of encephalitis, acute, probably Japanese B encephalitis. Serum was submitted in many cases, but since the illness was so short, no significant changes were found. The important clinical findings apparently were bizarre, instant neurological signs, clouding of the sensorium and fever with death in coma. Of

Table XLI. Duration of Disease From Recorded Onset to Death in
Fatal Japanese B Encephalitis

<u>Days</u>	<u>No. of Cases</u>	<u>Days</u>	<u>No. of Cases</u>
1	2	8	0
2	4	9	0
3	7	10	3
4	2	11	1
5	8	12	0
6	6	13	1
7	2	92	1
		Total	37*

* In 2 cases, the date of onset is unknown

the 33 clinically recognized cases, tissue for virus isolation was received in 31 instances. In one case the specimen was lost in transit. Isolates identified as Japanese B encephalitis virus were obtained in 13 of the 31 attempted isolations. In 38 cases, histologic sections showed the characteristic changes of Japanese B encephalitis. In the one case with a long interval between onset and death (92 days) sections of the nervous system were not typical of Japanese B encephalitis, and the diagnosis was made on clinical and serological findings. As might be expected, attempts to isolate a virus were unsuccessful in this case, but it is included in all of the tables pertaining to virus isolation given below.

Virus Isolation - As seen in Table XLIII this study indicates that the time between death and autopsy is not a significant factor in successful virus isolation within a 20 hour limit. Table XLIV suggests that there is at least another feature of importance, namely, the duration of illness before death. It appears that isolation of the virus is likely to be more successful if there is a clinical duration of disease of less than 6 days.

Table XLIII. Relation Between Results of Virus Isolation Attempts
and the Time Elapsing Between Death and Autopsy

<u>Time from Death to Autopsy, in Hours</u>	<u>Positive Virus Isolation</u>	<u>Negative Virus Isolation</u>
Less than 1 hour	1	3
1 - 2	3	7
2 - 3	0	0
3 - 4	4	1
4 - 5	0	1
5 - 6	0	2
6 - 7	0	0
7 - 8	1	1
8 - 9	0	2
11 - 12	2	0
18 - 19	2	0
25 - 26	0	1

The plan to compare the results of frozen specimens with specimens kept in buffered glycerin had to be abandoned. However, it should be pointed out that simple transportation is of little importance since virus isolates were obtained in as high a percentage of specimens from Korea as from Japan (7 of 18 specimens from Korea, or 39% and 6 of 13 specimens from Japan, or 46%).

Table XLIV. Relation Between Results of Virus Isolation Attempts and Duration of Disease

<u>Duration of Disease in Days</u>	<u>Positive Virus Isolation</u>	<u>Negative Virus Isolation</u>
1	2	0
2	2	0
3	4	3
4	1	1
5	3	4
6	0	6
7	0	1
10	0	1
11	1	0
12	0	1
92	0	1

Autopsy Findings - Gross examination of the brain showed little except marked congestion and increase in weight of the brain. In the one prolonged case, there was definite but moderate cerebral atrophy. The microscopic changes were typical of those described in the literature (4, 5, 6). They fell into a pattern readily recognizable with a scanning lens. There was a mild meningitis with mononuclear cells predominating. In many sections, there was prominent perivascular cuffing, varying in intensity from mild to very severe. Although there was considerable variation from case to case and section to section, all showed typical small nodular accumulations of cells in the cerebral and cerebellar cortex and in various cranial nuclei. The cells in such nodules were mainly mononuclear, although in places polys were also present. The mononuclear cells have not been positively identified although we presently suspect a microglial origin. In some of these nodules, glitter cells were present supporting this opinion. The ground substance in such nodules was frequently broken up and apparently degenerating. Neurones entrapped in such nodules frequently were undergoing lysis, degeneration, and neuronophagia. Other neurons, only partly entrapped, appeared viable. Although there was considerable variation in the intensity and frequency of this inflammatory reaction, certain nuclear masses appeared to be constantly involved. These included the inferior olivary nuclei, the nuclei pontis, the substantia nigra, the dentate nucleus and the thalamus. In those cases where sections of the spinal cord were available, the inflammatory changes were generally intense and diffuse and probably not to be differentiated of themselves from poliomyelitis, although both the anterior and posterior horns were generally involved and the reaction sometimes spilled over into the lateral column.

There was one other change found so far in 17 of the 39 cases. This consisted of focal round to oval areas of encephalomalacia. They varied greatly in size, number, and distribution. In some cases they appeared to fuse. Generally, they were without cellular reaction and suggested areas of sudden acute anemic infarction. However, a search for obstructed vessels was unsuccessful. More intensive study will be required to learn what the basic process and the significance of such lesions are. Table XLV shows the relation of the presence of these lesions to the duration of the disease. Apparently this process is more likely to appear in cases of shorter duration, but not all cases of short duration show this change. Table XLVI suggests that recovery of the virus and the presence of foci of encephalomalacia are related in some way. However, it is not known whether one determines the presence of the other. Other factors such as age, time between death and autopsy, and time between autopsy and animal inoculation, were not significantly different for cases with or without foci of encephalomalacia.

Encephalitis and War Wounds - There were 9 patients who were hospitalized because of war wounds who subsequently died with Japanese B encephalitis. Of these 9 patients, 3 were recognized clinically and virus isolation was successful in 2. These 3 patients had the following injuries: (1) A relatively minor war wound of a foot, with death ascribed to Japanese B encephalitis; (2) a moderately serious war wound of the face with contusion of one temporal lobe, the onset of symptoms of Japanese B encephalitis being preceded by a clear period during which the prognosis appeared quite favorable.

Table XLV. Presence of Foci of Encephalomalacia in Relation to Duration of Disease Prior to Death

<u>Duration of Disease in Days</u>	<u>Encephalomalacia Present</u>	<u>Encephalomalacia Absent</u>
1	1	1
2	2	2
3	5	2
4	1	1
5	5	3
6	3	3
7	0	2
10	0	2
11	0	1
12	0	1
13	0	1
92	0	1
Total	17	20

(Duration unknown in 2 cases)

Table XLVI. Relation of Presence of Foci of Encephalomalacia and Isolation of Virus

	<u>Positive Virus Isolation</u>		<u>Negative Virus Isolation</u>	
	No.	%	No.	%
Encephalomalacia present	10	59	7	41
Encephalomalacia absent	3	21	11	79

Death was believed due to Japanese B encephalitis rather than to the war wound; virus isolation was successful; (3) a serious war wound of the abdomen with intestinal perforation and peritonitis. Prognosis for the war wound was considered guarded but not hopeless. The patient developed symptoms of Japanese B encephalitis and died the following day. Virus isolation was successful. Death was believed due to the combination of peritonitis and Japanese B encephalitis. The histologic sections showed severe central nervous system involvement.

The remaining 6 patients with war wound and Japanese B encephalitis had severe head wounds. The clinical course was similar in all cases. A diagnosis of Japanese B encephalitis was not made or suspected. However, in each case it was recognized that the war wound did not explain the clinical signs and symptoms. In these cases, subdural or subarachnoid hemorrhage, brain abscess, or meningitis was suspected and burr hole explorations were performed to find sites of hemorrhage. In these cases, the clinical signs and symptoms were suspicious in retrospect, and in each case the histologic changes were characteristic of Japanese B encephalitis. In none of these was there an attempt made to isolate the virus of Japanese B encephalitis.

Because of the limited number of cases involved, it is not possible to state unequivocally that patients with Japanese B encephalitis and with brain injury due to battle injury have a poorer prognosis than patients with only one of these conditions. However, comparison with non-fatal cases of Japanese B encephalitis suffering battle injuries does support such an opinion. There were 31 patients who survived battle injuries and were diagnosed clinically, and in most cases by confirmatory laboratory tests, as having Japanese B encephalitis. Only one of these may have had brain injury. Table XLVII lists the battle injuries in this group. The extremity wounds varied from a gunshot wound of a finger to wounds of multiple extremities of a degree to require amputation.

Table XLVII. Battle Injuries and Japanese B Encephalitis

<u>Injury</u>	<u>No. Fatal</u>	<u>No. Non-Fatal</u>
Head with brain damage	7	0
Concussion	0	1
Scalp	0	1
Extremity and Scalp	0	2
Extremities	1	22
Thorax and Extremity	0	1
Abdomen	1	1
Abdomen and Spine	0	1
Unknown	<u>0</u>	<u>2</u>
Total	9	31

The higher fatality rate for Japanese B encephalitis with battle injury (22.5%) than without battle injury (10.6%) may be statistically significant, but the large number of known variables involved indicate that a mathematical study of the results is not warranted. Thus, it is suspected that at least several of the head injury cases would have died as a direct result of the head trauma. It is known that during the time of the epidemic battle casualties in some cases were evacuated to the Z.I. within 24 or 48 hours after reaching Japan. Subsequently, some of these may have developed Japanese B encephalitis. A few cases could change the percentage results a great deal. It is hoped that the study of the intensity and distribution of lesions of Japanese B encephalitis in the fatal cases may assist in answering these questions. In the case with mild trauma of the temporal lobe, the preliminary study suggested that the lesions of Japanese B encephalitis were more intense in the region of trauma. Thus, there may be a relation between brain damage and intensity of tissue involvement in Japanese B encephalitis.

The date of onset of symptoms of Japanese B encephalitis in patients with battle injuries was determined in 7 cases. In 5 of these cases, these determinations were made in retrospect from the clinical records and may not be accurate. Therefore, a comparison of the duration of illness of patients who died with Japanese B encephalitis, with or without battle injury, must be made with caution. Omitting the prolonged case (92 days duration) and patients with battle injuries, the average duration of symptoms in the fatal cases of Japanese B encephalitis was 4.8 days. The average duration of symptoms of Japanese B encephalitis in patients with battle injuries was 6.1 days. The mean duration for both groups was 5 days.

The one prolonged case was in a patient who developed the typical signs and symptoms of Japanese B encephalitis and appeared to be recovering, only to relapse into a vegetative state. He died from severe malnutrition. Serial complement fixation tests showed a rise to a 4-plus reaction in a dilution of 1:8 followed by a fall to negative. Histologic sections showed almost no activity. Virus isolation attempt was unsuccessful. Further study will be required in this case.

Yoshida Sarcoma - Early in November 1950, two white rats bearing Yoshida Sarcoma were obtained by this laboratory. In view of the Korean conflict, it was apparent that experimental work with the neoplasm would have to be limited largely to a morphologic study of the disease process and a familiarization with techniques involved in its study. Any further investigation was to be postponed until additional facilities and technical assistance were available.

Historical - Yoshida Sarcoma was first discovered in an albino rat fed o-aminotoluene by Professor Yoshida at Nagasaki, Kyushu, Japan in 1943 (7). Rats which were subsequently inoculated intraperitoneally with ascitic fluid from the original animal developed an ascites in which there were large numbers of tumor cells apparently undergoing rapid proliferation; thus Yoshida chose to call the disease an ascites sarcoma. This tumor apparently is similar in many respects to the Ehrlich Ascites tumor of mice (8).

The tumor was readily transmitted from one rat to another by intraperitoneal injection of ascitic fluid from a diseased animal. The rats succumbed to the disease quite regularly in approximately 10 days. It was soon suggested that this represented an excellent experimental device for determining the effectiveness of various chemotherapeutic agents used in the treatment of neoplastic disease. Further work on the tumor demonstrated its close morphologic relationship to monocytic leukemia. Considerable work has been done on the changes in the disease when animals other than the original strain of albino rats are used.

Preliminary Studies - Thirteen albino rats of the strain originally used by Yoshida were inoculated intraperitoneally with ascitic fluid from tumor bearing rats. The average survival after inoculation was 10 days. Ten were autopsied and histologic preparations were made. All but 2 of the rats had a pronounced ascites with marked abdominal distention at the time of death. The abdominal fluid contained large numbers of tumor cells. All animals exhibited relatively large, firm, friable, light gray or light yellow tumors of the greater omentum. These omental tumors were occasionally hemorrhagic. Grossly detectable invasion of the mesentery, retroperitoneal tissues, and epididymis was present. Frequently, small whitish or light gray deposits of tumor tissue were found on the inferior surface of the liver and along the margins of the spleen. No gross lesions were noted in the lungs, pericardium, or mediastinum. A small tumor was found routinely in the abdominal wall at the site of needle puncture.

Microscopic examination of the tissue removed at autopsy revealed widespread invasion of the omentum, mesentery, epididymis, retroperitoneal fat, pancreas, and skeletal muscle of the abdominal wall with wide separation and pressure atrophy of normal components of the various tissues. Thin deposits of tumor were present on the liver capsule, splenic capsule, and testicular capsule. The tumor often partially or completely surrounded the adrenal glands and kidneys but had not penetrated their capsules. Small clusters of the neoplastic cells were evident in the liver sinuses or along small veins in the liver. They were also seen in the spleen, and rarely in the lungs.

The cells were relatively large with a pale eosinophilic, poorly delineated cytoplasm and polygonal or stellate outline. Their nuclei were of moderate size, having an oval, round or indented outline with a distinct nuclear membrane and a finely reticulated chromatin pattern. Large basophilic or amphophilic nucleoli were prominent. Numerous mitoses were seen. In the larger tumor masses, extensive degeneration and necrosis was found.

Smears of ascitic fluid regularly showed large numbers of the tumor cells which closely resembled monocytes of human blood. Blood smears prepared from heart blood drawn at autopsy or just prior to death revealed that these cells had entered the peripheral blood in all cases. Four animals were sacrificed, one each at 24, 48, 72, and 96 hours after inoculation so that the earliest stages of the disease process could be studied.

Ascitic fluid from 2 rats was lyophilized, reconstituted after a short period of time, and used to inoculate several rats. None of these rats developed the disease although all succumbed after inoculation with fresh ascitic fluid from tumor bearing animals.

One rat was inoculated subcutaneously and the resulting tumor was excised on the thirteenth day. The animal survived and was reinoculated subcutaneously on two occasions without development of a grossly detectable tumor mass at the site of inoculation.

Liaison with Other Laboratories - Close relations were maintained with the various hospitals in the Far East Command during the ordinary course of providing pathological diagnosis on submitted specimens. During the June meeting of the Military Surgeons of Japan, a program for a tumor board and for follow-up of patients returned to the Z.I. was proposed. Later in the same month, a visit was made to the hospitals in Southern Japan, during which conferences were held on professional subjects. It was intended to repeat such trips at regular intervals. The outbreak of hostilities in Korea disrupted these plans. However, even closer liaison occurred, because officers assigned to these hospitals as Laboratory Officers had previously been assigned to the Pathology Department, 406th Medical General Laboratory.

A method for distributing protocols and material for clinico-pathological conferences was organized. At the end of the year, these were being distributed to four hospitals in Japan outside of the Tokyo Area. In addition, CPC's were presented weekly for two of the hospitals and surgical conferences weekly at one of the hospitals by members of this department.

In August 1950, the 406th Medical General Laboratory established an advance section at the 118th Station Hospital in Kyushu (ADSEC 406th MGL). A pathologist was assigned from the Pathology Department. In addition to his duties with the ADSEC, he served as a pathologist for that hospital.

The usual liaison was maintained with the AFIP. During 1950, available histological slides, paraffin blocks, wet tissues, and protocols were forwarded from 469 autopsies and 1,447 surgicals. Comments and additional information obtained from the AFIP were sent to the originating stations. During the last three months of the year, there was an increased demand for study sets provided by the AFIP. The orthopedic and general surgery study sets were in constant use during this time. Other sets have been requested from the AFIP.

TRAINING ACTIVITIES: Medical Officers placed on duty with the 406th Medical General Laboratory to await reassignment were given an orientation course, during which they spent time in all departments of the laboratory. Emphasis was placed on the facilities available at this laboratory, the problems they were likely to meet, the procedure necessary for submitting various types of material for definitive examination, and the type and amount of information required to accompany such material. Most of these officers came from civilian and army residencies and knew very little of military channels and procedure. Further, some were not familiar with the forms and information required by the AFIP. Depending on the time available, these officers worked up at least a few autopsies submitted by contributing hospitals. The relation between contributing hospitals and a histopathological center was explained.

Intra-departmental conferences were held twice a week. One consisted of a slide conference on interesting cases seen that week and, when time permitted, this was followed by a journal club meeting. The other was a clinico-pathological conference in which the pathologists acted as clinicians and the true diagnosis was known only to one officer of the staff. This conference, held one evening a week, proved very popular and sometimes lasted for more than two hours.

At various times, formal lecture courses in pathological techniques and basic pathology were given to enlisted technicians. Due to the pressure of work, these were short-lived. Continuous on-the-job training was substituted.

OFFICER PERSONNEL: During 1950 a total of 24 medical officers were assigned to the Pathology Department. The four officers present for duty at the beginning of the year returned to the Z.I. prior to 1 June. Three of the 5 replacements were serving in Korea by 1 August, two of whom were serving with infantry divisions.

The 21 medical officers on duty with the Pathology Department for varying periods of time after 1 June served as a pool for laboratory officers. By the end of the year, 15 had served with other organizations in Japan and Korea, two had just arrived, two were shortly to be returned to the Z.I., and only two spent more than five months on continuous duty in the department. Table XLVIII shows the assignment of these officers. Several of these officers served with more than one unit, e.g., with the mobile blood bank and then a hospital in Japan. Some of the officers on TDY in the theater returned to the Pathology Department for several weeks prior to returning to their home stations.

The 25 officers consisted of 2 who had passed their boards in pathology, 3 who had completed formal residency training, 8 who were in army residency, and 6 in civilian residency training programs when they were ordered to the Far East Command. Three officers had made application for army residency training in pathology.

Eighteen of the 25 officers were members of the Regular Army and 1 of the Regular Air Force. Three were Naval Reserve officers and 3 were Army Reserve officers. Of the latter 3, 2 returned to the Z.I. prior to 1 June and 1 had an application pending for Regular Army commission.

Table XLVIII. Reassignment of Officers (1 January - 31 December 1950)

Returned to Z.I. prior to 1 June	4
Reassigned to 25th Inf. Div., Korea	2
Reassigned to 8217th Mob. Med. Lab., Korea	3
Reassigned to 8054th Evac. Hosp., Korea	1
Reassigned to 8216th Mob. Med. Lab., Japan	1
Reassigned to hospitals in Japan	6
Reassigned to Mobile Blood Bank, Japan	1
Reassigned to ADSEC, 406th MGL, Japan	1
Not reassigned	6
Total	25

DIFFICULTIES ENCOUNTERED: As was true for all department in the laboratory, the Pathology Department was handicapped by insufficient space. Although the work load increased, the officer and secretarial staff was doubled and storage and shipping requirements were tripled, available space was decreased because one of the three rooms had to be shared with the Medical Zoology Department. The office formerly considered just adequate for 4 officers and a secretary contained 6 officers and 2 secretaries at the end of the year.

An even greater problem was secretarial help. The 2 secretaries could not keep pace with the large number of reports, correspondence, filing, and routine office administration. At the end of the year, a backlog of 115 completed autopsies was waiting to be typed. Attempts to obtain follow-up information and additional information for the AFIP had to be curtailed. Dictation was kept to a minimum and the officers submitted most of their work in long hand for final form typing.

Although the large and often rapid turnover of officer personnel placed a burden on the department, this was not considered a difficulty. The orientation and training given these officers resulted in work and reports of such a highly satisfactory type when they were subsequently reassigned that the effort required was more than repayed. This was especially evident when the work of these officers was compared with that of laboratory officers who had not been assigned to this Laboratory. In addition, liaison was much closer when the laboratory officers were known personally.

Facilities for medical illustration, particularly of microscopic sections, were inadequate. Efforts are being made to improve this situation.

RECOMMENDATIONS: The 1949 Annual Report recommended that the "Standard Form 515" (Tissue Examination) be changed so that a space will be provided for recording the service number of personnel of the Armed Forces. This recommendation is repeated, with the added recommendation that such a space should be used for the serial number of the military person dependent upon when the surgical specimen is obtained from a civilian dependent.

Experience with the large number of officers assigned to the department, observation of their work after reassignment to other organizations, and comparison of their work with that of officers with similar training who had not had temporary assignment at this laboratory, emphasized certain deficiencies in current training policies. These deficiencies were more apparent in officers coming from civilian residencies, but were also found among officers coming from army hospitals. Generally, no criticism could be made of the professional training given. However, few of the officers were acquainted with military channels and procedures. They were not familiar with the importance of establishing line of duty status and the question of homicide, suicide, or accident when performing autopsies. They had not seen medical supply catalogues and did not know how to obtain supplies. They were unfamiliar with military correspondence and the channels for returning reports.

Although well trained as clinical and anatomical pathologists, they did not know the capabilities, facilities, and responsibilities of a general laboratory, an army laboratory, a histopathological center, and higher echelons.

Although this list of deficiencies seems large, in practice it was found that education along these lines could be accomplished in a short time. Because the results were so satisfactory, it is recommended that all laboratory officers be assigned for a one or two week period to the nearest army laboratory and histopathological center for training along the lines indicated. It might also be suggested that Chiefs of Laboratory Service in army hospitals having residents be informed of the fact that residents are not getting this minimal training in necessary military administration.

DEPARTMENT OF SEROLOGY

Routine: Exclusive of investigative and Blood Bank work, 113,171 routine procedures were completed in 1950 (Table I). This basic figure is an increase of 1.5% over 1949. However, consideration of serologic procedures performed as the laboratory phase of Blood Bank operation results in a 71% increase in total serologic procedures performed.

Table I. Routine Serologic Procedures

Cardiolipin Microflocculation Tests (Qualitative)...	80,685
Cardiolipin Microflocculation Tests (Quantitative) ..	8,566
Cardiolipin Complement-Fixation	13,564
Pandy	1,968
Colloidal Gold	1,969
Heterophile Agglutination	3,095
Cold Agglutination	393
Blood Grouping	729
Rh ₀ Typing	701
Rh ₀ Antibody Titration	1,315
Total	<u>113,171</u>

Standard Tests for Syphilis - As reported previously (1) this department performs all STS for medical installations in the Tokyo-Yokohama area including 1 General Hospital, 6 Station Hospitals, 2 large Central Dispensaries, and others. In addition, reactive sera are still forwarded from all other medical installations in Japan for confirmation, quantitative test, and complement-fixation. Cardiolipin is the antigen used in all Army installations now. With the onset of the Korean war, departure of troops, and discontinuance of the serologic check on the venereal disease program, the monthly average of 11,770 tests dropped to 7090 (plus 3670 related to Blood Bank). Realizing that these total examinations do not constitute a random sample, it appears worthwhile to report the degree of positivity in the larger groups (Table II).

Table II. Cardiolipin Positivity

	<u>American Military</u>	<u>American Civilian</u>	<u>Japanese</u>	<u>Other Nationals</u>
No. Tested	76,360	3,142	837	54
No. Positive	4,111	139	82	11
% Positive	5.4	4	10	22

Rh Factor - Limited amounts of anti-Rh serum other than Rh₀ were received, allowing more definitive work in this field. Of 1,315 antibody titrations on maternal Rh negative blood, incomplete antibodies were demonstrated on 86 occasions. During the course of the year there were 3 cases of the Rh iso-immunization complex. One of the patients with a "blocking antibody" titer of 1:4096 was of value when Blood Bank operation at the start of the Korean war depleted theater stocks of typing serum. Despite continued search, no instances of the rarer types of Rh sensitization have been uncovered. The press of markedly increased activities has prevented taking full advantage of the excellent opportunity for study of blood factors and antibodies available through Blood Bank processing.

Erythroblastosis in Japanese - An opportunity was presented through certain Japanese associates to perform Rh analysis in two suspect cases of erythroblastosis. The low incidence of Rh negative blood in Japanese has been previously recognized (2) and has been found in our own experience. The occurrence of Rh iso-immunization is an extreme rarity, only 2 previous cases having been observed (3). The first case studied showed the father and child to be CDE and the mother CDe. Maternal serum gave no evidence of complete or incomplete antibodies with CDE, Cde, and cde cells. Coombs test was likewise negative. The second case studied gave the following history of 6 pregnancies:

First: Terminated by spontaneous abortion
 Second: Neonatal jaundice with survival
 Third: Jaundice on second neonatal day with death on fifth day
 Fourth: Neonatal death with jaundice
 Fifth: Neonatal death with jaundice
 Sixth: Induced abortion with autopsy diagnosis of erythroblastosis fetalis

Rh typing revealed the father to be cDE, the child cDe, and the mother cdE. Maternal serum showed no titer for saline antibodies, but anti-D incomplete antibodies in a titer of 1:1024.

Preservation of Sheep Erythrocytes (4) - As reported previously (1, 6) the feasibility of prolonged storage of sheep cells as a further attempt at reducing variables in the complement-fixation test and other serologic procedures has been under study in this department for the past few years. The most promise was shown by a dextrose-gelatin-veronal (DGV) solution. Through continued experimentation, it was found that a 2% suspension of sheep cells could be preserved for periods exceeding one month by the following method.

One liter of DGV containing the following constituents was prepared:

Sodium barbiturate	0.38 gm.
Calcium chloride, anhydrous ...	0.02
Magnesium sulfate (7H ₂ O)	0.12
Sodium chloride	8.50
Dextrose	10.00
Barbituric Acid	0.58
Gelatin	0.20

The barbituric acid and gelatin were dissolved in 150 ml. each of boiling distilled water. These were then added to 700 ml. of an aqueous solution containing the other reagents. Subsequent autoclaving at 15 pounds for 30 minutes showed little or no carmelization, but the solution usually required adjustment to pH 7.6. Sheep blood was collected aseptically in modified Alsever's solution (7, 8). Using sterile precautions a pooled 50 ml. total was removed, equal portions centrifuged in two 50 ml. cups and both sediments washed three times with 40 ml. each of DGV. Washed cells were combined and resuspended to approximately 2.5% in a measured quantity of DGV. Next 0.2 ml. of this suspension was laked with 1.8 ml. of distilled water, the OD of the diluted hemoglobin determined spectrophotometrically, and suspension adjusted to an OD of 0.400 $\pm$ 0.01. This resulted in a 2% suspension containing 500,000 RBC per mm³.

Preserved cell suspensions were periodically studied to determine hemolysis, cell count, hematocrit, MCV, osmotic fragility, pH, and sterility. Suspensions were also subjected to test of reactivity in the heterophile agglutination test (9) in parallel with fresh suspensions (Table III). A comparison of reactivity of cells preserved up to 12 weeks with those of fresh suspensions is shown in Table IV.

Preservation of Sensitized Sheep Cells for Complement-Fixation Test (5) -

Using the same solution and methods described above with larger amounts to allow production of 2.5 to 3 liters of 2% cell suspensions, equal volumes of the standardized cell suspension and optimally diluted amboceptor in DGV were mixed in two large containers by pouring back and forth repeatedly. Such rapid and massive mixing was found to achieve the most satisfactory sensitization. The sensitized suspension was kept at room temperature for one hour prior to normal refrigerator storage at 4°C. Extensive comparative testing of such suspensions with fresh cells and those preserved in modified Alsever's solution and in MgSO₄-saline showed excellent preservation and reactivity to immune hemolysis.

Table III. Comparison of Heterophile Agglutinin Titrations Performed in Parallel with Fresh and DGV-Preserved Suspensions

<u>Sera* Tested</u> <u>(No.)</u>	<u>Endpoint¹</u>	<u>Agreement²</u> <u>(No.)</u>	<u>Disagreement</u> <u>(No.)</u>
43	Undiluted	43	0
89	7	89	0
150	14	150	0 ^x
102	28	101	1 ^x
46	56	45	1 ^x
29	112	29	0
25	224	25	0
34	448	34	0
20	896	20	0
4	1792	4	0
3	3584	3	0
3	7168	3	0
1	14336	1	0
549		547	2

* Sera were tested with suspensions prepared daily and with suspensions preserved for periods up to 12 weeks.

¹ The endpoint represented the reciprocal of the highest dilution giving at least 1⁺ macroscopic agglutination.

² An endpoint difference of one dilution was considered essential agreement.

x These sera showed endpoints which differed by two dilutions from the endpoints obtained with fresh suspensions.

Table IV. Comparison of Heterophile Agglutinin Titrations Performed in Parallel with Fresh and with DGV Suspensions Preserved for Varying Periods of Time

<u>Preserved</u> <u>in DGV</u> <u>(weeks)</u>	<u>Sera Tested</u> <u>(No.)</u>	<u>Agreement*</u> <u>(No.)</u>	<u>Disagreement</u> <u>(No.)</u>
1	73	73	0
2	69	69	0
3	48	48	0
4	57	57	0
5	45	45	0
6	70	70	0
7	36	36	0
8	49	48	1 ⁺
9	20	20	0
10	32	31	1 ⁺
11	19	19	0
12	31	31	0
Total	549	547	2

* An endpoint difference of one dilution was considered essential agreement.

⁺ These sera showed endpoints which differed by two dilutions from the endpoints obtained with fresh suspensions.

BLOOD BANK

Prior to the onset of hostilities in Korea certain few hospitals in Japan, qualified to engage in definitive surgical care, collected blood from their respective donor lists for use on specific cases in accordance with AR 40-1715. Tokyo Army Hospital and Osaka Army Hospital maintained small blood banks in amounts sufficient to meet their own needs. On 3 July, it became apparent that participation of U. S. forces in the Korean conflict required an immediate implemented plan for centralized control and supply of whole blood in large amounts. The 406th Medical General Laboratory was given the mission of preparing and operating such an installation. From personnel assigned to other duties in this laboratory a unit was formed for this purpose. At a later date (17 August 1950) the 8090th Blood Bank Laboratory Detachment was organized as a TD unit attached, and subsequently assigned, to the 406th Laboratory for this specific mission.

The 406th Blood Bank was organized originally to consist of: (a) a Collecting and Processing Center in Tokyo; (b) an Advance Blood Bank Depot at Fukuoka; (c) a Transportation and Courier Section. Meanwhile Blood Bank Sections were being activated as an organic part of Medical Supply Depots in Korea. On 7 July, actual blood bank operations began and that night the first shipment of 69 bottles of "O" blood was made direct to the 8054th Evacuation Hospital which was opening in Pusan on that date. Special railway cars were designed and equipped to provide an initial means of transporting blood to the Advance Blood Depot. Later airlift was utilized, except when weather conditions required reversion to the originally prepared methods. Air Force cooperation in such shipments has been outstanding in routine and all emergency instances.

Considering the size of the potential donor panel in the Tokyo-Yokohama area, it was felt that local procurement could guarantee no more than 100 pints of blood per day. Policy at that time prohibited the use of Japanese donors, but, fortunately, donations were received in adequate amounts from non-combat troops of Army, Air Force, Navy, and Allied Forces, civilian employees of the Armed Forces, commercial entrants, foreign nationals other than Japanese, and adult dependents of these groups. Local Red Cross agencies furnished a volunteer corps to assist in donor procurement and donor care. Approval having been received, a Japanese donor program was integrated on 28 September.

According to Mason (10) the British Blood Transfusion Service with the Middle East Command reported a ratio of 1 pint of blood to 10 wounded as adequate whereas U. S. forces in the Mediterranean theater required 1 pint per 2.2 wounded. In neither case is it absolutely clear whether this constitutes actual usage of blood, or the amounts needed in Theater supplies to allow for the loss between base blood bank and patient. In our own experience the supply for the first five weeks was on a "hand-to-mouth" emergency basis as troop strength rapidly built up in Korea, and more field medical installations were formed and sent in to care for casualties.

By the end of this brief period, making due allowance for the necessary establishment of depot stocks, an attempt was made to determine whether a firm ratio could be found to allow sensible and required future planning. Using Theater G-1 figures which were available on a rapid, accurate, daily basis, it became apparent that the ratio of pint of blood to wounded in action held fairly constant at .82 pints per WIA. Using projected combat strengths, and general factors for estimating battle casualties (11) an educated guess of casualties could be made and interpreted in terms of blood requirements.

This long term estimate of blood requirements made it obvious that the available donor panel could not hope to meet the maximum needs, and request was accordingly made for gradually increasing amounts to be shipped from the Zone of the Interior. In general it appeared logical to attempt to adjust the amounts from this source by timely forecasts, and to use local sources to meet emergency needs. In addition local procurement would allow supply of group and Rh specific blood to hospitals in Japan, since shipment of blood other than group "O" from the U. S. did not appear practical or economically feasible.

This original estimate has undergone numerous revisions as is to be expected from any forecast dependent on day-to-day battle losses. In the present war the extremes of favor and disfavor meeting our forces have necessarily required continuous and close attention to tactical reports and estimates of enemy capabilities to avoid periods of too little blood and also those to too much. Experience of 6 months has also shown a gradually increasing ratio of requirements of blood in terms of wounded in action (Fig. 1). It should be realized that the present 3.32 per WIA represents an experience factor for logistic planning of theater blood requirements. This includes usage and also the unavoidable waste that accompanies storage and distribution of such a perishable product.

It is regretted that maintenance of security prevents presentation of detailed data in an unclassified report such as this, but it is hoped that the above may be of value in demonstrating a method of planning and operating a centralized theater blood supply service.

Japanese Donors - A realistic composite of standards of several recognized authorities (12, 13, 14) were prescribed as the original basis for donor interview and acceptance. With the integration of Japanese donors certain new factors were introduced in addition to those of language difficulty. The latter required hiring a minimal Japanese staff plus nurses and volunteers supplied by the Japanese Red Cross. One problem concerned a reluctance by Japanese Medical Authorities to depart from their standard practice of limiting blood donations to 200 ml. per bleeding. Tables of maximum collection were prepared for bleeding Japanese and others of small stature (Table V). Using these standards there has been no evidence of harmful effects, immediate or delayed, in such donors.

Table V. Amount of Blood to be Collected per Pound of Body Weight from Japanese Nationals and other Donors of Small Stature

Male				Female			
Body Lbs.	Weight Kgs.	Blood To Be Collected (ml.)	Anticoagulent & Blood Together (ml.)	Body Lbs.	Weight Kgs.	Blood To Be Collected (ml.)	Anticoagulent & Blood Together (ml.)
100	45	250	370	105	48	230	350
105	48	260	380	110	50	240	360
110	50	275	395	115	52	250	370
115	52	290	410	120	55	260	380
120	55	300	420	125	57	275	395
125	57	310	430	130	59	285	405
130	59	325	445	135	61	295	415
135	61	330	450	140	64	310	430
and more		maximum	maximum	145	66	320	440
				150	68	330	450
				and more		maximum	maximum

During the period 28 October to 30 December blood has been collected from 2,784 Japanese donors. As a preliminary report on the racial characteristics of this sample from the standpoint of major blood group and Rh factor, findings are shown in Table VI.

It becomes apparent from the following table (Table VI) that the low incidence of Rh negative blood limits to a great extent the large scale use of Japanese donors if Rh compatible blood is to be given to a recipient population composed in large part of American and European nationals.

Figure 1 Ratio of Blood Requirements
Pints per WIA

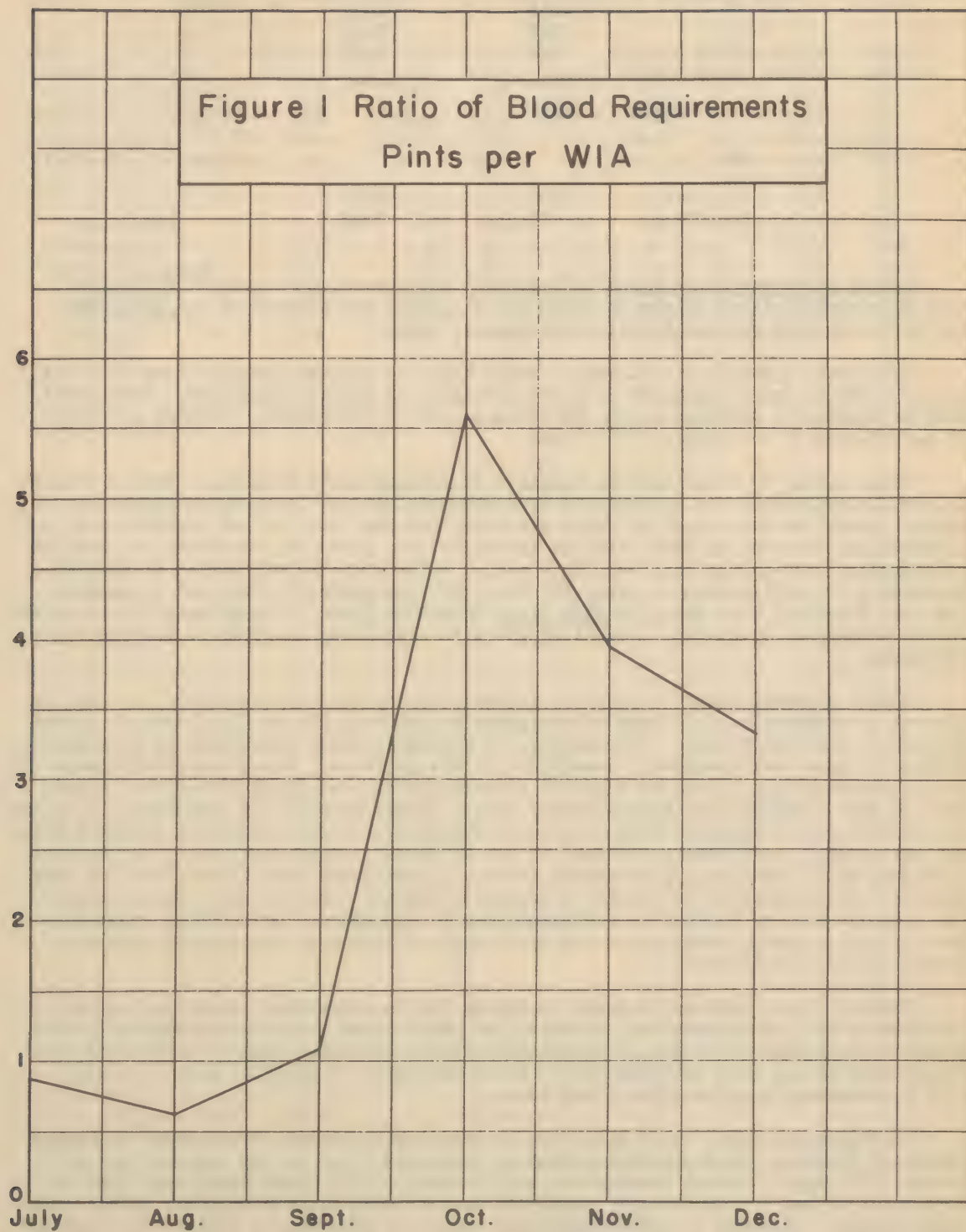


Table VI. Incidence of Major Blood Groups and Rh Factor in Japanese

<u>Group</u>	<u>Number</u>	<u>Percentage</u>
O	912	32.75
A	1015	36.45
B	618	22.19
AB	239	8.58
Total	2784	

Rh

Positive	2765	99.32
Negative	19	0.68

Use of Preserved Whole Blood - Faced with the necessity of general dissemination of recommendations on use of blood the following was formulated and published in the Eighth Army Medical Bulletin for August, 1950.

"The 406th Blood Bank will supply whole blood to medical installations in Korea and to those in Japan concerned with the treatment of battle casualties. Such blood will be labeled to indicate group, Rh factor, date of collection, serology and titer of agglutinins in the case of 'O' blood.

"Only group 'O' blood will be supplied to medical units in Korea. When practical in such installations the suitability of a specific unit of blood for a particular recipient should be determined by cross-matching, but when this is not feasible due to difficulties inherent in field medical installations, group 'O' blood may be given as an emergency life-saving measure. Only meager authoritative information is available concerning the utilization of group 'O' blood of high antibody titer but in general, and when feasible, such blood of high titer should be given to recipients who are themselves members of group 'O'. Low titer group 'O' blood may be given to individuals of any group.

"While thorough check to avoid Rh incompatibility would be desirable, in most instances determination of Rh type of recipient is not feasible in field medical installations in the combat zone. Fortunately, an extremely small percentage of Rh negative males will have been previously sensitized to the Rh factor, hence the chief damage will lie in sensitization of the Rh negative individuals who are given Rh positive blood. Even in such circumstances approximately only 1 person in every 25 instances will exhibit any evidence of sensitization. In those soldiers who have previously received blood and may possibly have been sensitized to the Rh factor, administration of Rh positive blood may still serve as a life-saving measure in emergency conditions since the reaction to such transfusion is usually a delayed hemolysis. Accordingly, the necessity for determination of Rh type in recipients can be disregarded under field conditions. Entry should be made, however, on the field medical record of the specific group and type of blood administered.

"Medical installations in Japan receiving battle casualties requiring further transfusion will be expected to determine the major blood group and Rh type of recipients to allow administration of compatible blood. Cross-matching of blood will be a requirement in all such installations. Blood according to specific group and type will be obtained from the 406th Blood Bank.

"Although preserved whole blood can be kept satisfactorily for use for a maximum period of 30 days, the expiration period of preserved blood at the present time will be set at 21 days. Visual examination will be made of all blood drawn more than 10 days prior to use for evidence of hemolysis as indicated by pink staining of the overlying plasma. Evidence of marked hemolysis (deep red staining of plasma) will warrant destruction of such a bottle of blood."

Although some may argue the suitability of certain portions of the above directive, it has proven practical and adequate judging from reports received from using units. A review of data included in reports from using units is presented in Table VII.

Table VII. Use of Blood in Korea 7 July-15 November, 1950

Reporting Unit	Clr. Co.	MASH	MASH	MASH	EVAC HOSP	EVAC HOSP	FIELD HOSP	HOSP SHIP
Days of Operation	41	117	112	10	150	55	44	59
Units Received	48	1600	1311	112	980	64	800	2402
Units Used	9	1550	1043	45	960	52	600	1235
Patients Transfused	6	?	435	34	394	50	400	386
No. Reactions	0	3	10	1	5	1	21	34
Hemolytic	0	(3)	(1)	0	(1)	0	(3)	(0)
Pyrogenic	0	0	(5)	(1)	(4)	0	(12)	(6)
Allergic	0	0	(4)	0	0	(1)	(6)	(28)
Units Discarded	39	50	268	18	20	0	200	304
Over-age	(39)	(10)	(268)	(18)	0	0	(150)	(186)
Hemolysis	0	(40)	0	0	(6)	0	(30)	(118)
Clots	0	0	0	0	(14)	0	(20)	0

DEPARTMENT OF ENTOMOLOGY

Since the activation of an Entomological Section in this laboratory in 1949, intensive studies on the role of mosquitoes and other insects in the epidemiology of Japanese B encephalitis have been undertaken. These studies have consisted of mosquito and fly population surveys, studies on various phases of mosquito biologies which bear upon disease transmission, and investigations to determine which arthropod species carry the virus of Japanese B encephalitis in nature. In addition, this section has provided routine insect and arthropod identifications incident to the operation of a medical general laboratory and has provided such other entomological services as are required for the support of medical and other installations.

The work performed showed an overall increase of approximately 500% as compared with the previous year (Table I).

Table I. Summary of Activities - Department of Entomology

Adult mosquito identifications	519,201
Larval mosquito identifications	596
Fly identifications	5,015
Other insect identifications	31
Mosquito blood precipitin tests	5,000
Virus isolation tests	560*
Total	530,403

* An additional 800 virus isolation tests were conducted jointly by the Virus and Rickettsial Department and the Department of Entomology during the winter of 1949-1950

INSECT STUDIES IN RELATION TO JAPANESE B ENCEPHALITIS: Introduction - As in 1949, a survey was undertaken in Tokyo during the mosquito breeding season to determine mosquito species population relationships with the Japanese B encephalitis epidemic and epizootic, and to obtain material for virus isolation tests. A total of 514,761 adult mosquitoes were collected in this survey. Of this number, 158,817 mosquitoes were sealed in 2,010 tubes, quick frozen and preserved on dry ice for virus isolation tests. In addition, 105 lots containing 9,423 fresh mosquitoes were turned over to the Virus and Rickettsial Department for immediate attempts at virus isolations during the summer.

As an adjunct to the mosquito studies, a survey was undertaken of the biting stable fly, Stomoxys calcitrans. This species, which shows a high biting propensity for man, horses, cattle, swine, and other animals was considered to meet many of the requirements of a vector species. Collections of Stomoxys totalled 5,015 of which number 4,455 were frozen and preserved for subsequent virus isolation tests. An additional 474 Stomoxys were turned over to the Virus and Rickettsial Department for immediate attempts at virus isolation during the summer. Two other species of flies were taken in small numbers during the survey, and a few specimens of one, Tabanus mandarinus, were frozen for virus isolation attempts.

Mosquito populations were studied throughout the season by means of adult resting station collections, light trap collections, animal bait trap collections, and by human biting collections. Every effort was made to maintain collections throughout the breeding season on an unvarying basis so that the observed results would closely reflect population changes. A summary of all adult mosquitoes collected during 1950 in Tokyo has been prepared in Table II. This tabulation includes all mosquitoes collected during routine population studies as well as studies on special projects, exclusive of the overwintering survey.

Table-II. Summary of All Adult Mosquitoes Collected During 1950 in Tokyo

Species	Resting Station Collect.	Light Trap Collect.	Bait Trap Collect.	Human Biting Collect.	Totals
<u>Anopheles hyrcanus sinensis</u> Wied.	2,352	5,753	2,387	52	10,544
<u>Anopheles sineroides</u> Yamada	0	2	1	0	3
<u>Armigeres obturbans</u> Walk. = <u>A. subalbatus</u> (Coq.)	165	503	3,124	221	4,013
<u>Tripteroides bambusa</u> Yamada	1	0	0	0	1
<u>Uranotaenia bimaculata</u> Leic.	5	0	0	0	5
<u>Aedes vexans nipponii</u> (Theob.)	655	6,228	4,559	32	11,474
<u>Aedes albopictus</u> (Skuse)	46	5	130	256	437
<u>Aedes togoi</u> (Theob.)	283	19	34	32	368
<u>Aedes nipponicus</u> La C. & Yamag.	0	0	28	3	31
<u>Aedes japonicus</u> (Theob.)	1	1	19	0	21
<u>Culex tritaeniorhynchus</u> Giles	17,324	299,593	117,675	1,115	435,707
<u>Culex pipiens</u> Linn.	44,425	1,658	2,446	3,186	51,715
<u>Culex bitaeniorhynchus</u> Giles	26	186	94	16	322
<u>Culex hayashii</u> Yamada	67	3	5	0	75
<u>Culex mimeticus</u> Noe	7	1	1	1	10
<u>Culex orientalis</u> Edw.	1	0	0	0	1
<u>Culex vorax</u> Edw.	32	0	0	0	32
<u>Culex whitmorei</u> (Giles)	0	0	1	0	1
<u>Culex sinensis</u> (Theob.)	0	1	0	0	1
Totals	65,390	313,953	130,504	4,914	514,761

ADULT RESTING COLLECTIONS: Twenty-four adult resting stations were established scattered throughout Tokyo. One station consisting of pig pens had to be dropped in mid-season when the owner sold his pigs. Collections were made once weekly from these stations, with the objective of collecting all mosquito adults found at rest. The period of collections extended from 14 May to 30 September.

A summary of collection data by type habitat is given in Table III. This data shows little variation from the results obtained in 1949 (1). As in the previous year, animal shelters such as dairies, stables, and pig pens yielded heavy catches of Culex tritaeniorhynchus, while houses, caves and chicken houses showed a preponderance of Culex pipiens. Of all types of mosquito resting habitats, a single shed produced the largest mosquito yields, 98% of which were Culex pipiens. Among animal shelters, dairy barns yielded the largest catches of Anopheles hyrcanus, Armigeres obturbans = (A. subalbatus), Culex tritaeniorhynchus, and Culex pipiens. As in 1949, collections of resting mosquitoes in houses showed a very heavy preponderance of Culex pipiens. For every specimen of Culex tritaeniorhynchus taken in houses, 166 specimens of Culex pipiens were collected.

A comparison of mosquito resting station collections in urban and rural areas in 1950 also shows little variation from the results obtained in 1949 (1). Table IV, which summarizes the results obtained in 1950, indicates larger catches of Anopheles hyrcanus, Armigeres obturbans, Aedes vexans, and Culex tritaeniorhynchus from rural stations. Culex pipiens was taken in slightly larger numbers in rural stations also, but the results in 1949 had previously indicated a slight preponderance in urban stations. In rural stations, C. tritaeniorhynchus constituted 37% of the total mosquito catch, as contrasted with only 6% in the urban stations. In 1949, collections of Culex tritaeniorhynchus were even more heavily weighed toward the rural stations.

Population curves of three mosquito species based on the average number of mosquitoes taken per resting station collection are given in Figure 1. Collections of Culex pipiens were larger than those of any other species, with Culex tritaeniorhynchus being second most numerous. The C. pipiens population reached a high level on 18 June and remained high for four weeks, with the peak occurring during the week of 25 June. The C. tritaeniorhynchus population progressed much more slowly, reaching a peak during the week of

Table III. Resting Station Collections by Type Habitat

Habitat Type	Hab. No.	Average per Collection						Totals		No. Col-lections
		<u>A. hyrcanus</u>	<u>A. obturbans</u>	<u>A. vexans</u>	<u>C. pipiens</u>	<u>C. tritaen.</u>	Misc. spp.	No. Col-lected	Ave. per Col-lection	
Horse stables	4	8.3	0.3	4.5	20.01	72.3	0.7	8,169	106.1	77
Dairy barns	5	15.8	1.4	3.01	72.07	88.7	0.5	17,236	181.4	95
Pig pens	3	4.6	0.02	0.2	46.0	67.4	0.3	5,337	118.6	45
Chicken houses	4	0.0	0.0	0.0	49.4	2.0	0.2	4,118	51.5	80
Houses	4	0.01	0.01	0.01	61.3	0.4	1.03	4,765	62.7	76
Sheds	1	0.2	0.1	0.6	670.9	4.1	3.0	12,220	678.9	18
Caves and bomb shelters	3	0.01	0.08	0.05	229.1	0.6	3.7	13,545	233.5	58
Totals	24	5.2	0.4	1.5	98.9	38.6	1.04	65,390	145.7	449

Table IV. Mosquito Collection from 12 Urban and 12 Rural Resting Stations

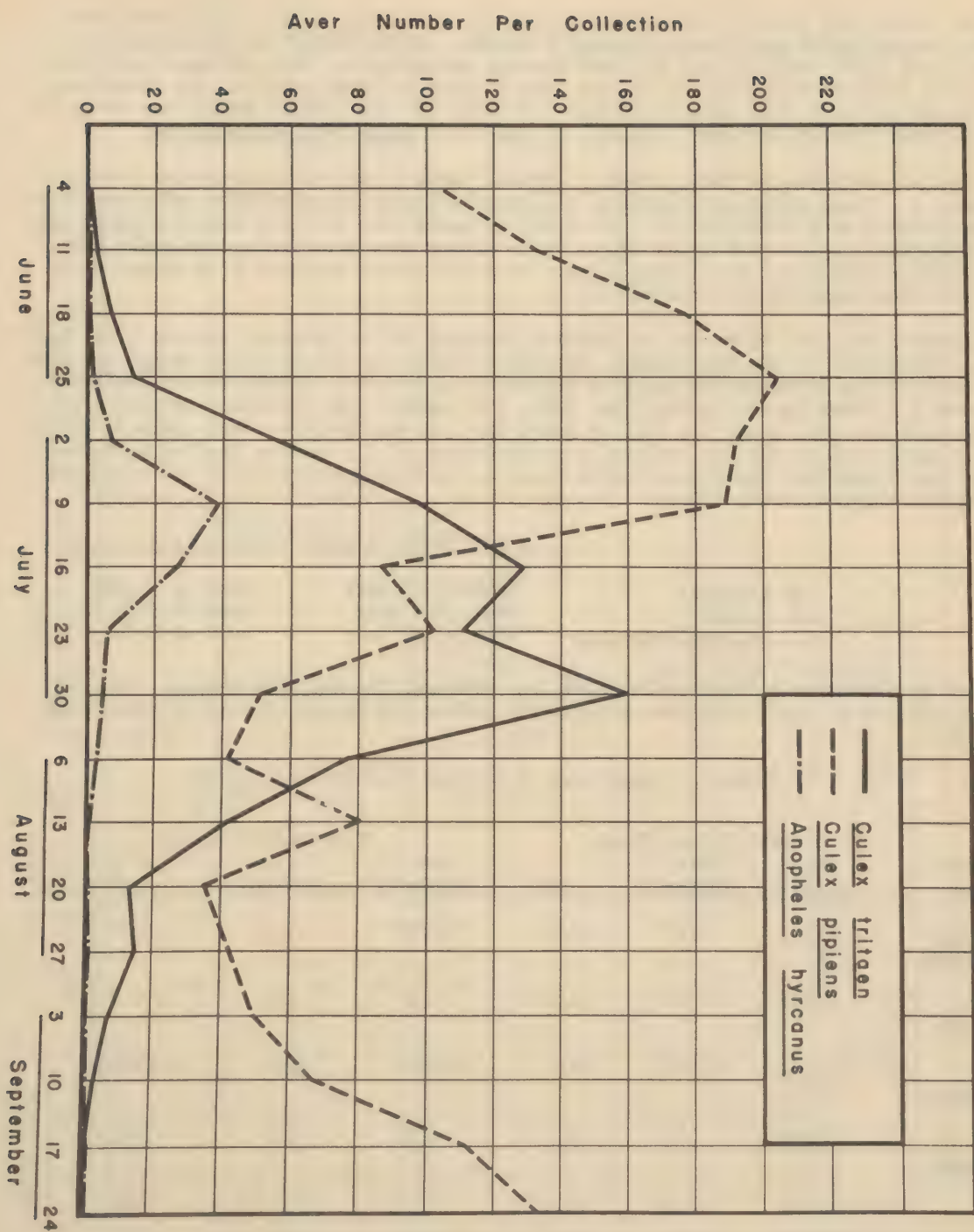
Species	Urban		Rural		Totals	
	No. Col-lected	% Col-lected	No. Col-lected	% Col-lected	No. Col-lected	% Col-lected
<u>A. hyrcanus</u>	49	0.1	2,303	5.3	2,352	3.6
<u>A. obturbans</u>	8	0.1	157	0.4	165	0.3
<u>A. vexans</u>	223	1.0	432	1.0	635	1.0
<u>C. tritaen.</u>	1,325	6.0	15,999	37.2	17,324	26.5
<u>C. pipiens</u>	20,439	91.3	23,986	55.8	44,425	67.9
Misc. Spp.	335	1.5	134	0.3	469	0.7
Totals	22,379	100.0	43,011	100.0	65,390	100.0

30 July. Population trends and peaks during the 1949 mosquito survey showed good correlation by the various methods of collecting employed. However, the results obtained in 1950 by adult resting station collection differed from the results obtained by light trap collection, animal bait trap collection, and human biting collection. A discussion of the deviations in results obtained by the several methods and of their significance is presented later in this report.

LIGHT TRAP COLLECTIONS: During the period of 20 May to 30 September, five mosquito light traps were operated thrice weekly as an additional method of measuring mosquito population trends. Due to difficulties with the electric current supply the schedule of operations was difficult to maintain, resulting in some variation in the number of trap-nights obtained from week to week. The location of the five traps were as follows:

1. A Japanese type residence in Honchodori.
2. An American military housing area in Pershing Heights.

Fig. 1 Population Curves Based on Resting Station Collections - Tokyo, 1950



3. A horse stable in Yoyogi.
4. The Tokyo Metropolitan Police stables.
5. A dairy in Shimotakaido.

All traps were located out of doors, with the exception of the trap at the Tokyo Police stables, which was operated inside a stable. At the height of the mosquito season, this light trap yielded 35 times as many mosquitoes as the next most productive trap. The huge catches obtained in this trap account in large part for the startling differences in light trap catches in 1949 and 1950. Of the 177,335 mosquitoes taken in routine light trap collections, 169,122, or 95%, were Culex tritaeniorhynchus.

Population curves of the 3 mosquito species taken in largest numbers are presented in Figure 2. These curves are based on the average number of mosquitoes taken per light trap collection on a weekly basis. It should be noted that the peak week for Culex tritaeniorhynchus was the week of 23 July. This differs from the findings by adult resting station collections, but agrees closely with the curves obtained from animal bait traps and from human biting collections.

Location of light traps has an important bearing on the catches obtained from these traps. Comparison of two bait traps, one placed inside the Tokyo Police stable and the other outside the Shimotakaido dairy is presented in Table V. Comparison in catches is made over a 15 week period during June, July, and August, and represents 43 trap-nights of operation for each trap. It will be noted that the yields from these traps differed both in volume and in the proportional representation of the various species collected. It was also found that population curves based on collections from these two traps varied considerably, as shown below:

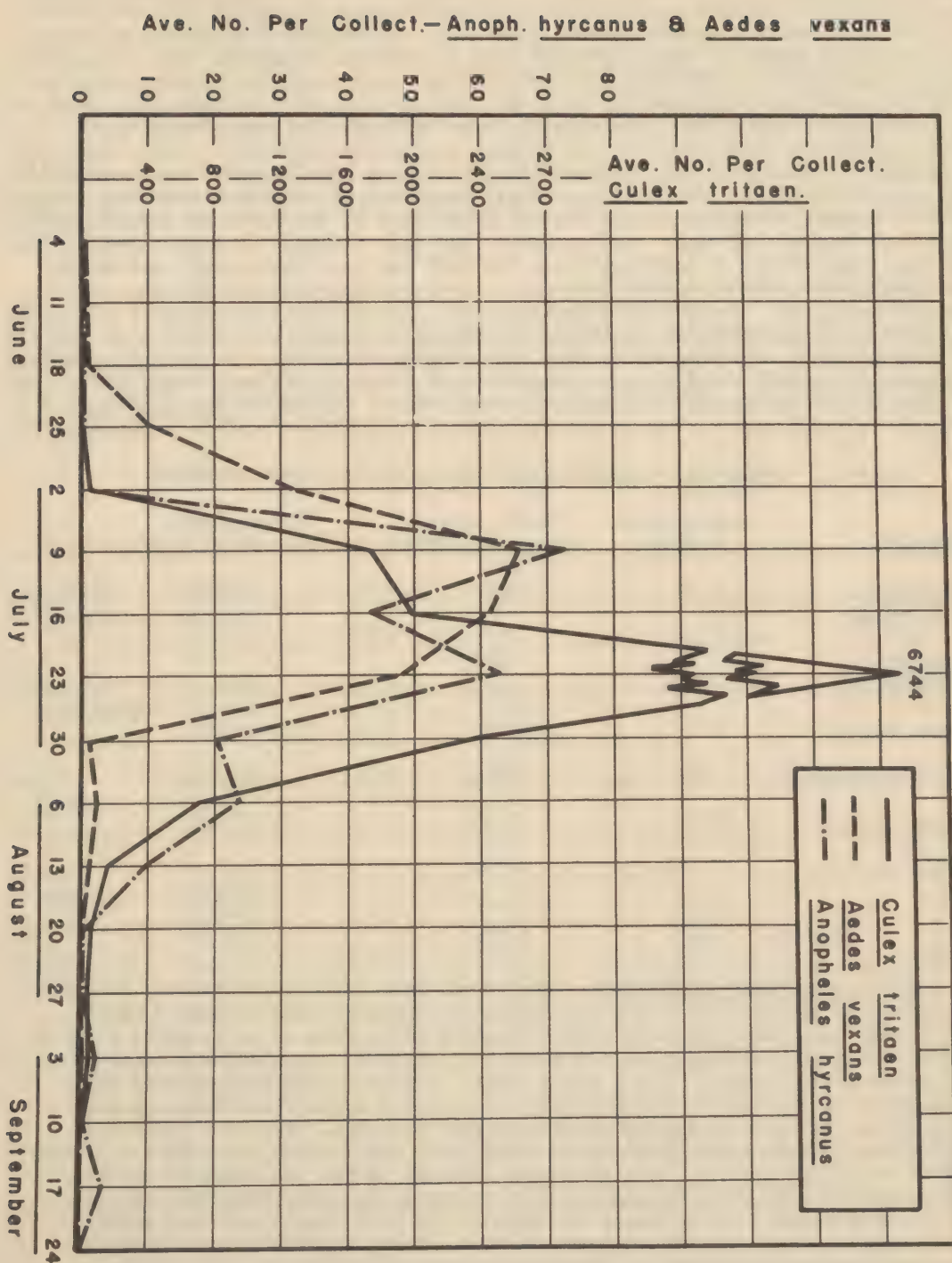
	<u>Tokyo Police Stable</u>	<u>Shimotakaido Dairy</u>
A. <u>hyrcanus</u>	week of 23 July	week of 9 July
A. <u>vexans</u>	week of 9 July	week of 2 July
C. <u>tritaeniorhynchus</u>	week of 23 July	week of 9 July

Inasmuch as the Tokyo Police stable light trap produced the heaviest catches, the overall computation of light trap data was heavily influenced by the results obtained from this trap.

Table V. Comparison of Catches from Two Light Traps

Mosquito Species	Tokyo Police Stable			Shimotakaido Dairy		
	No. Collect.	Ave. Collect.	%	No. Collect.	Ave. Collect.	%
<u>Anopheles hyrcanus</u>	1,144	27	0.7	2,275	53	12.2
<u>Aedes vexans</u>	2,892	67	1.8	437	10	2.3
<u>Culex tritaen.</u>	152,362	3,543	97.0	15,483	360	83.1
<u>Culex pipiens</u>	556	13	0.4	435	10	2.3
Misc. spp.	25	0.6	0.1	19	0.4	0.1
Totals	156,979	3,651	100	18,649	433	100

Fig. 2 Population Curves Based on Light Trap Collections—Tokyo, 1950



Light traps were used in a special study as a method of determining the effectiveness of window and door screening at the Yoyogi stables. Three light traps were utilized by placing one inside an unscreened stable, another inside a screened stable, and a third outside the stable. The two stables were separated by about 50 feet and were identical in structure. Each housed twelve horses. All three light traps were operated simultaneously for three nights during the week of 23 July. The results are indicated in Table VI. The reasons for the gross differences obtained in the three traps are not as obscure as would first appear. The discrepancy between catches from the trap outside the stables and the trap inside the unscreened stable were consistent with results pointed out earlier, i.e., the markedly larger catches from the trap located outside the Tokyo Police stable over the catches obtained from all other traps. The apparent explanation is that the effectiveness of light traps is greatly enhanced when traps are placed directly in a mosquito feeding situation. While the trap placed inside the screened stable yielded only 7% as many mosquitoes as the trap inside the unscreened stable, the yield was still large enough to indicate that protection against mosquitoes are not complete. An inspection of the screened stable indicated that all windows and doors were screened, but that a number of small cracks and crevices were uncovered. A small vent near the roof was also unscreened, and carelessness in closing doors also must have been a contributing factor. The importance of uncovered cracks and crevices in permitting the ingress of mosquitoes into buildings is further indicated by the magnitude of catches in animal bait traps. As will be indicated later in this report, catches from a single bait trap frequently number in the thousands after a single night of operation. These animal bait traps are nothing more than small screened structures with a baffle opening of one-half inch width, which is comparable to the cracks and crevices sometimes found in buildings.

Table VI. Comparison of Light Trap Catches in and Around Stables

<u>Species</u>	<u>Trap Outside Stables</u>	<u>Trap Inside Screened Stable</u>	<u>Trap Inside Unscreened Stable</u>
<u>Anopheles</u> <u>hyrcanus</u>	8	448	1,403
<u>Armigeres</u> <u>obturbans</u>	0	369	0
<u>Aedes vexans</u>	3	194	2,471
<u>Culex tritaenior.</u>	228	3,864	66,813
<u>Culex pipiens</u>	0	57	92
Misc. spp.	0	2	0
	—	—	—
Totals	239	4,974	72,779

ANIMAL BAIT TRAP COLLECTIONS: Six animal bait traps were operated during the 1950 mosquito survey. Two traps were operated at Yoyogi stable, each 3 nights weekly. In one trap, two pigs were used throughout the season as bait, while a horse was used as bait in the second trap. A third bait trap was operated at a dairy in Hatagaya three nights weekly, with a cow as bait. A fourth trap was operated three nights weekly at a pig farm in Jikeikai with two pigs as bait. This trap was taken out of operation in mid-season when the farmer sold his pigs. Two bait traps were operated at the Ueno Park Zoo four nights weekly with an alternation of horses, cows and pigs in the traps as bait. The operation schedule of the two traps at the Ueno Park Zoo provided three cow trap-nights, three horse trap-nights, and two pig trap-nights. In this manner, the original schedule of all bait trap operations provided for weekly runs of eight pig trap-nights, six horse trap-nights, and six cow trap-nights. With the discontinuance of the Jikeikai trap in mid-season, the number of pig trap-nights decreased to five. Animal bait trap operations commenced 11 June, terminating 30 September. Male mosquitoes were rarely taken, the catches being largely

engorged females regardless of the type of bait used. During the season, bait traps were operated a total of 271 trap-nights.

In routine operation of bait traps, population trends were measured by results obtained with horse, cow, and pig as bait. The population curves thus obtained for Culex tritaeniorhynchus showed differences in magnitude but good correlation otherwise. Curves for Culex tritaeniorhynchus based on the mean number of specimens taken per trap night by the various types of bait animals are given in Figure 3. Population curves in Figures 4 and 5 for Anopheles hyrcanus and Aedes vexans, where horse and cow were used as bait, show similar correlation. The number of A. hyrcanus and A. vexans obtained with pig bait was too small to construct population curves. Good correlation in population curves of Armigeres obturbans (A. subalbatus) was obtained from horse and pig baits, but the number of this species obtained from cow baited traps was too small for comparison (Figure 6). Population curves of Culex pipiens obtained from pig and cow baited traps were widely divergent, while the number of this species obtained from horse baited traps was too small for statistical comparison.

The relative attractiveness of horses, cows and pigs to the various mosquito species is illustrated in Table VII. From this table it will be observed that horses are the most attractive bait, of the animals tested, for Armigeres obturbans, Culex tritaeniorhynchus, and for total numbers of all mosquito species. Cows serve as the most attractive hosts, of the three types of host animals used, for Anopheles hyrcanus and Aedes vexans, while the largest number of Culex pipiens were found in pig baited traps.

Table VII. Summary of Animal Bait Trap Collections

Species		Horse	Cow	Pig	Total
<u>Anopheles hyrcanus</u>	No. Coll.	1,137	803	42	1,982
	No. per				
	Trap-Night	10.2	11.0	.5	7.3
<u>Armigeres obturbans</u>	No. Coll.	2,418	113	526	3,057
	No. per				
	Trap-Night	21.8	1.5	6.0	11.3
<u>Aedes vexans</u>	No. Coll.	1,747	1,981	216	3,944
	No. per				
	Trap-Night	15.7	27.1	2.5	14.6
<u>Culex pipiens</u>	No. Coll.	105	631	1,654	2,390
	No. per				
	Trap-Night	.94	8.6	19.0	8.8
<u>Culex tritaeniorhynchus</u>	No. Coll.	56,879	33,735	13,341	103,955
	No. per				
	Trap-Night	512.4	462.1	153.3	382.6
Misc. spp.	No. Coll.	195	49	50	294
	No. per				
	Trap-Night	1.7	0.6	0.6	1.1
Totals	No. Coll.	62,481	37,312	15,829	115,622
	No. per				
	Trap-Night	562.9	511.1	181.9	426.6
No. Trap - Nights		111	73	87	271

Fig. 3 Culex tritaeniorhynchus Population Curves Based on
Animal Bait Traps Tokyo, 1950

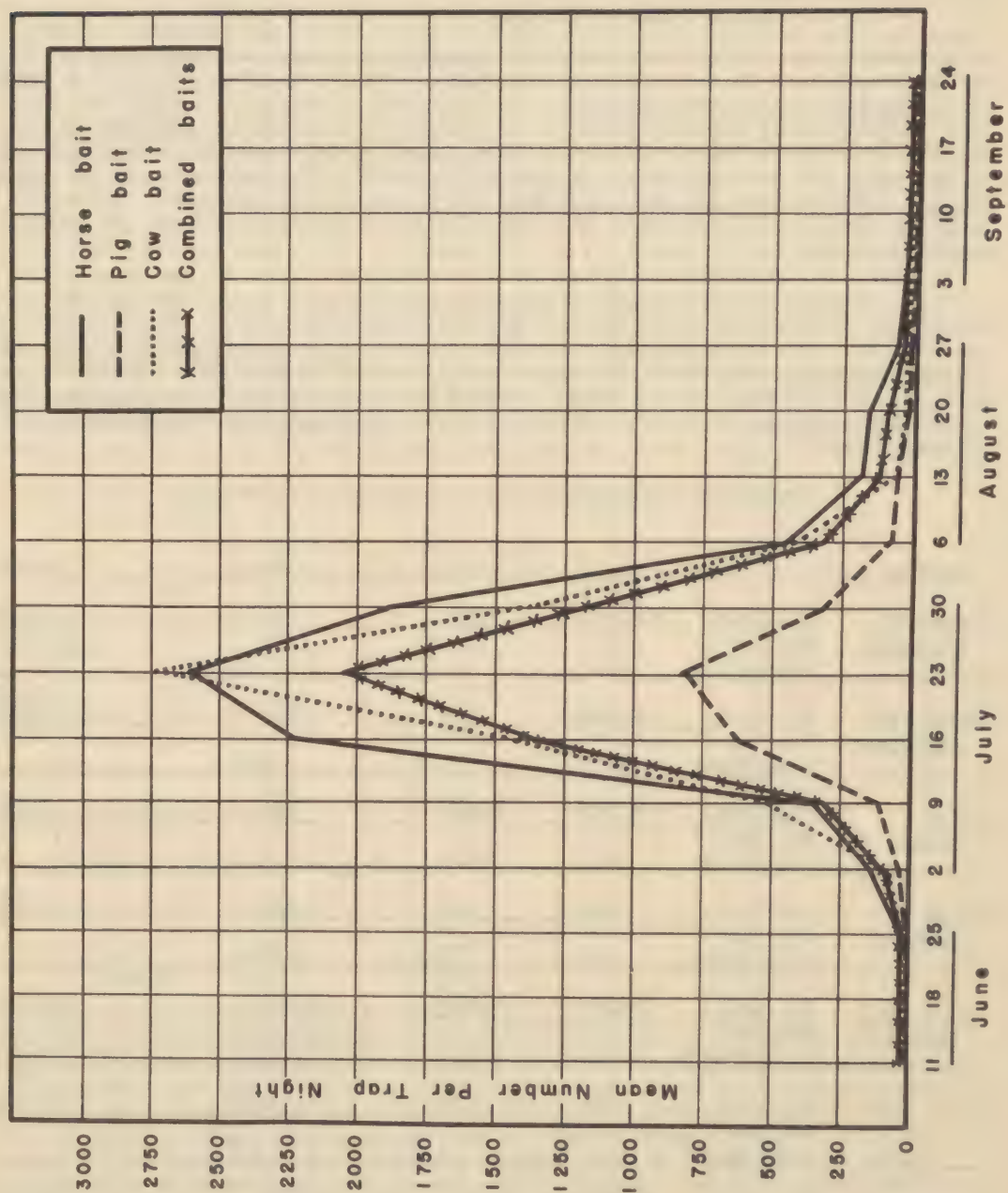


Fig. 4 Bait Trap Collection Rates of Anopheles hyrcanus

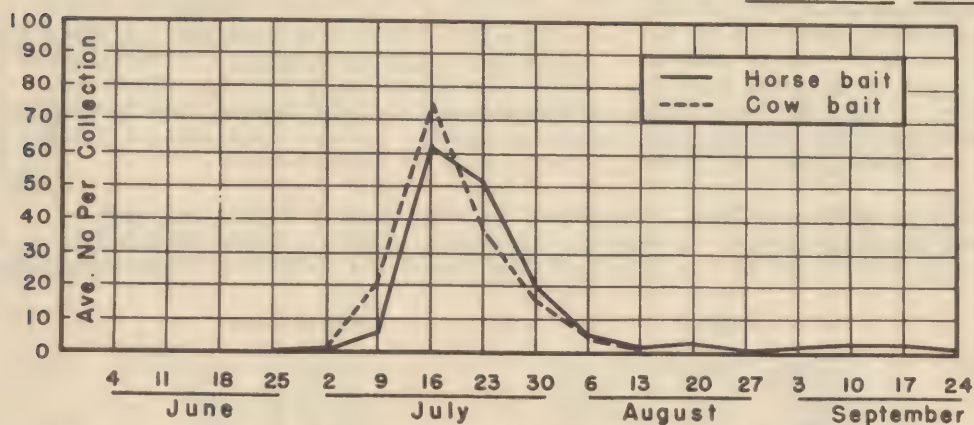


Fig. 5 Bait Trap Collection Rates of Aedes vexans

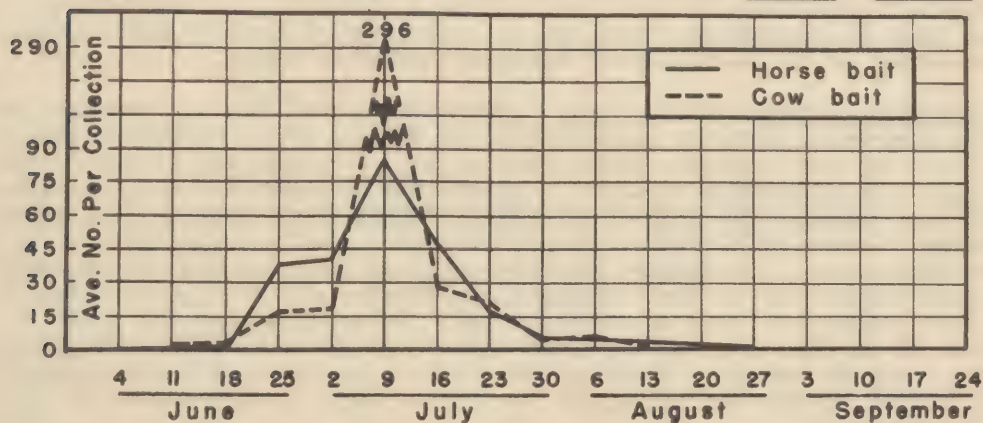
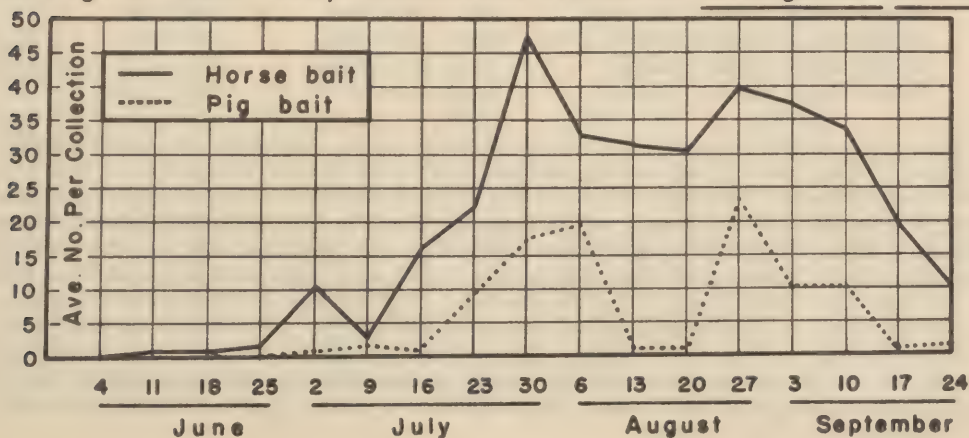


Fig. 6 Bait Trap Collection Rates of Armigeres obturbans



In previous studies of mosquitoes by the animal bait trap method in Japan (1), (2), (3), (4), investigators have centered trapping activities in a single location. The use of bait traps in four different locations during this survey permitted a comparison of catches in varied localities where the same host animal was used as bait. All traps were located within a radius of 6.8 miles in the city of Tokyo. It was found that considerable variation occurred in traps separated by short distances. Illustrative of these differences are the data assembled in Tables VIII and IX. From these tables it can be noted that variation occurred not only in the magnitude of the catches but also in the proportional representation of the various mosquito species. In traps at Hatagaya Dairy and at the Ueno Park Zoo, where cows were used as bait, marked variation was noted in catches of Anopheles hyrcanus sinensis. Similarly, at Yoyogi stable and at the Ueno Park Zoo, where horses were used as bait, disproportionate catches of Armigeres obturbans were obtained. In both tables, the variation in catches of Culex tritaeniorhynchus should be noted. Similar variation in catches was obtained at three locations where pigs were used as bait in traps.

Bait traps in different localities where the same type of animal was used as bait sometimes showed variation in mosquito population trends. Traps baited with horses at Yoyogi stable and at the Ueno Park Zoo showed the following population peaks:

<u>Yoyogi</u>		<u>Ueno Park</u>
<u>A. hyrcanus</u>	week of 23 July	week of 16 July
<u>A. vexans</u>	week of 9 July	week of 25 June
<u>C. tritaen.</u>	week of 23 July	week of 23 July

The variable results thus obtained from bait traps illustrate the necessity of using several traps in separate locations for the collection of accurate data on mosquito host trophisms or for measurement of mosquito population trends.

HUMAN BITING COLLECTIONS: During 1949, limitations on the number of personnel assigned to this department prevented accomplishment of a human biting collection program. Faced with the same personnel limitations in 1950, a program was formulated for obtaining large-scale collections through the cooperation of Japanese biology students in four Tokyo high schools. The program established routine weekly biting collections from four biology classes of 25 students each, with each student devoting 2 hours per week to these collections. Two classes consisted solely of boys and two of girls only. Under the general supervision of their teachers, students in each class made biting collections during specified hours on specified days. Collections were made immediately in front of the students' home in clear weather, and in front of an open window indoors during inclement weather. A map spotted with the locations of the students' homes, showed a wide distribution throughout the city of Tokyo. The schedule for collections was as follows:

Mejiro High School (Girls)	Mon. and Tues.	7-8 P.M.
Nichidai 3rd High School (Boys)	Tues. and Wed.	8-9 P.M.
Tokyo High School (Boys)	Wed. and Thurs.	9-10 P.M.
Hakuo High School (Girls)	Thurs. and Fri.	10-11 P.M.

Collections were picked up once weekly from each class. During the school vacation period from 25 July to 10 September, collections were mailed directly to the laboratory by each collector. Throughout the survey not a single male mosquito was turned in. Inasmuch as the collecting group was unacquainted with the fact that bloodsucking is restricted to female mosquitoes, it tends to verify the faithfulness of the collection methods employed. Throughout the season the level of time devoted to collections varied very little, approximating 175 hours weekly. During the entire season the total number of hours devoted to human biting collections was 2,593. The total number of mosquitoes taken in this survey was 4,914. The period of operation of this survey extended from 18 June to 30 September. Only a small percentage of the mosquitoes taken in this survey were engorged with blood. No differences were observed among the various mosquito species on this point.

Population curves for Culex pipiens and Culex tritaeniorhynchus are illustrated in Figure 7. Culex pipiens showed a marked increase in biting intensity in early July.

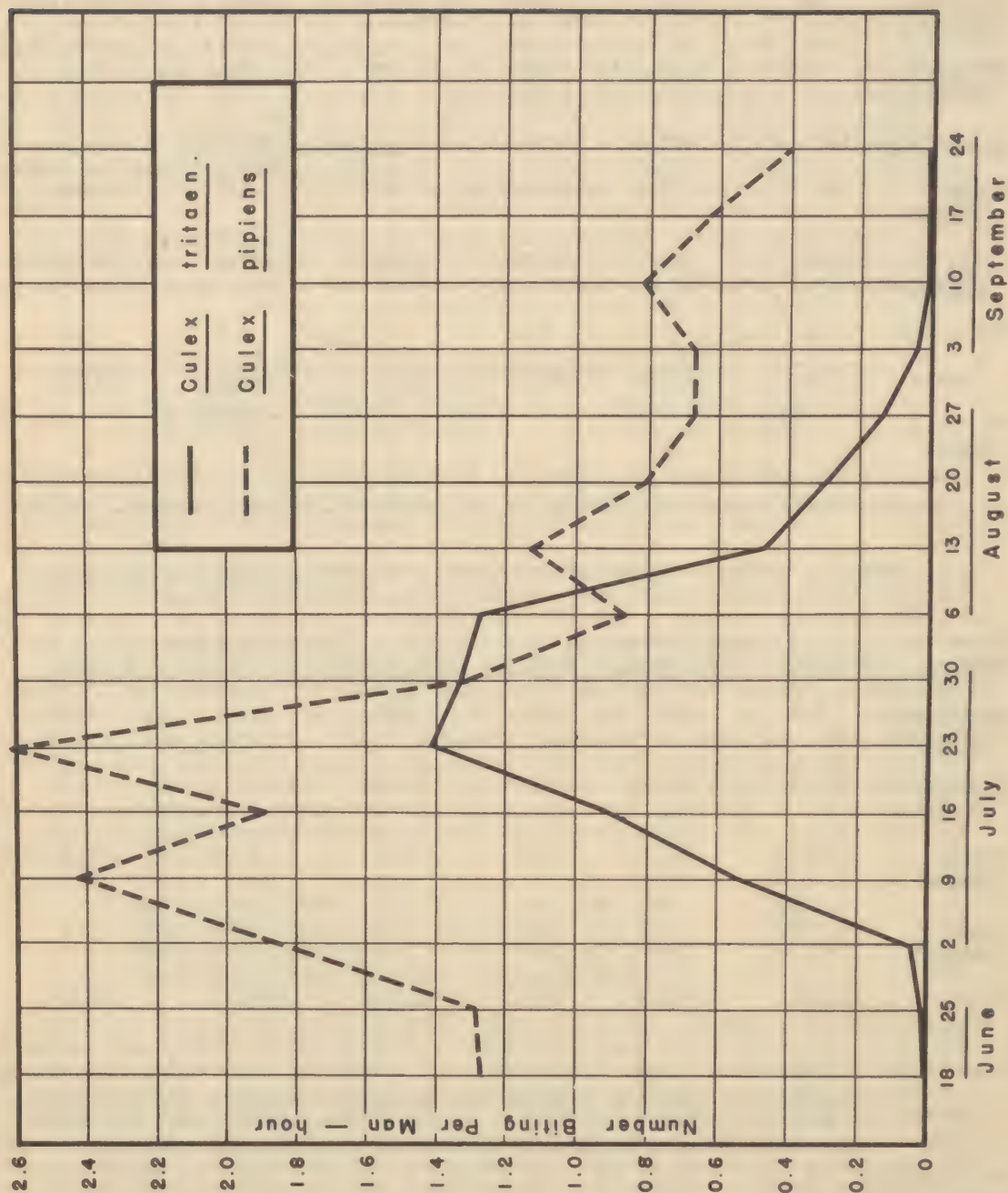
Table VIII. Effects of Locality on Animal Bait Trap Catches (Cow-bait)

Mosquito Species	Hatagaya Dairy			Ueno Park Zoo		
	No. Coll.	Ave./Coll.	% Coll.	No. Coll.	Ave./Coll.	% Coll.
<u>Anopheles hyrcanus</u>	764	21.2	2.6	39	1.1	0.5
<u>Armigeres obturbans</u>	6	0.2	0.1	107	2.9	1.3
<u>Aedes vexans</u>	1527	42.4	5.2	454	12.3	5.5
<u>Culex pipiens</u>	555	15.4	1.9	76	2.1	0.9
<u>Culex tritaen.</u>	26,210	728.1	90.1	7,525	203.4	91.5
Misc. Spp.	24	0.7	0.1	25	0.7	0.3
Totals	29,086	807.9	100.0	8,226	222.4	100.0
No. Trap-Nights		36			37	

Table IX. Effects of Locality on Animal Bait Trap Catches (Horse-bait)

Mosquito Species	Yoyogi Stable			Ueno Park Zoo		
	No. Coll.	Ave./Coll.	% Coll.	No. Coll.	Ave./Coll.	% Coll.
<u>Anopheles hyrcanus</u>	792	16.5	2.2	266	5.9	1.2
<u>Armigeres obturbans</u>	2,328	48.5	6.5	78	1.7	0.4
<u>Aedes vexans</u>	1,259	26.2	3.5	414	9.2	1.8
<u>Culex pipiens</u>	33	0.7	0.1	55	1.2	0.3
<u>Culex tritaen.</u>	31,292	651.9	87.4	21,590	479.8	95.9
Misc. Spp.	94	1.9	0.3	95	2.1	0.4
Totals	35,798	745.8	100.0	22,498	499.9	100.0
No. Trap-Nights		48			45	

Fig. 7 Population Curves Based on Human Biting Collections —Tokyo, 1950



dropped suddenly during the week of 16 July and reached a peak of biting intensity during the week of 23 July, after which it dropped rapidly. Culex tritaeniorhynchus similarly rose sharply in biting intensity in early July and progressed steadily until a peak was reached during the week of 23 July after which it dropped rapidly. The third most numerous species taken was Aedes albopictus, which showed a gradual build-up in biting intensity until it reached a peak on the week of 20 August, after which it gradually declined. The number of specimens of other mosquito species taken in the human biting survey was so small that population curves constructed for them would be of dubious value. An examination of weekly collection data with reference to time of sunset revealed no correlation.

Table X. provides a summary of human biting collection data broken down on an hourly basis. It should be noted that with the exception of Culex pipiens and Culex tritaeniorhynchus the volume of material is too small to be of value. With both C. pipiens and C. tritaeniorhynchus, no distinct time-of-biting trends are indicated. Inasmuch as different classes were responsible for collection of material at the different time intervals, any variations appearing in this table might be attributed to differences in the avidity of collection by the various groups, or possibly to differences in the areas in which collectors' homes were located.

Table X. Hourly Collection Rates in Human Biting Mosquito Survey

Mosquito Species	7-8 P.M.		8-9 P.M.		9-10 P.M.		10-11 P.M.		Totals	
	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.
<u>Anopheles hyrcanus</u>	5	.01	0	0	20	.03	27	.04	52	.02
<u>Armigeres obturbans</u>	85	.13	34	.05	77	.12	25	.04	221	.09
<u>Aedes albopictus</u>	104	.16	38	.06	84	.13	30	.04	256	.10
<u>Aedes togol</u>	18	.03	1	.01	11	.02	2	.01	32	.01
<u>Aedes vexans</u>	13	.02	2	.01	8	.01	9	.01	32	.01
<u>Culex tritaen.</u>	362	.55	107	.17	246	.37	400	.62	1,115	.43
<u>Culex pipiens</u>	764	1.15	445	.72	1,014	1.52	963	1.50	3,186	1.23
Misc. spp.	7	.01	0	0	13	.02	0	0	20	.01
Totals	1,358	2.05	627	1.01	1,473	2.22	1,456	2.26	4,914	1.00

Differences found in indoor and outdoor collections are summarized in Table XI. It should be noted that the collection rate (number collected per man hour) was higher outdoors for all species except Culex pipiens. However, the slightly increased collecting rate for this species indoors is of questionable importance since the difference is so small.

Table XI. Human Biting Collections Tokyo, 1950

Species	Outdoors		Indoors		Totals	
	No. Collected	No./Hr.	No. Collected	No./Hr.	No. Collected	No./Hr.
<u>Anopheles hyrcanus</u>	52	0.02	0	0	52	0.02
<u>Armigeres obturbans</u>	215	0.09	6	0.02	221	0.08
<u>Aedes albopictus</u>	241	0.1	15	0.06	256	0.09
<u>Aedes togoi</u>	31	0.01	1	0.004	32	0.01
<u>Aedes vexans</u>	31	0.01	1	0.004	32	0.01
<u>Culex tritaenior.</u>	1048	0.4	67	0.2	1115	0.4
<u>Culex pipiens</u>	2858	1.2	328	1.3	3186	1.2
Misc. spp.	20	0.008	0	0	20	0.007
Totals	4496	1.9	418	1.7	4914	1.9
Total No. Hours Coll.	2346		247		2593	

EVALUATION OF COLLECTION METHODS FOR MEASUREMENT OF MOSQUITO POPULATION: In our report on mosquito population studies in Tokyo during 1949 (1) it was emphasized that multiple collection methods were required for the accurate evaluation of mosquito population trends. This was evident from discrepancies found in the mosquito population curves obtained for various mosquito species by the several methods of collection employed. Repetition of these findings in 1950 reemphasize this point. However, mere emphasis on the observed discrepancies does not serve to clarify the problem of whether or not mosquito population trends bear a relationship to the occurrence of epidemics and epizootics of Japanese B encephalitis. Before conclusions can be reached on this question, some type of evaluation of the observed results must be made.

In Tables XII and XIII mosquito population peak dates are assembled from studies conducted in Tokyo during 1949 and 1950. Supplementing this are the dates obtained by Petrakis by human biting collections in Yokohama during 1949 (5). It can be noted that in 1949, correlation was found in the population peak dates for Anopheles hyrcanus and Culex tritaeniorhynchus.* In 1950, however, less correlation was evident in collections of Anopheles hyrcanus, while collections of Culex tritaeniorhynchus still maintained fairly close agreement. Aedes vexans collections which showed wide variation in 1949, produced excellent correlation during 1950.

Table XII. Mosquito Population Peaks in 1949

Mosquito Species	Resting Stations	Bait Traps	Light Traps	Larval Collect.	Yokohama ¹ Biting Collect.
<u>Anopheles hyrcanus</u>	24 July	24 July	24 July*	17 July	24 July
<u>Aedes vexans</u>	10 July	3 July	26 June	Not Collected	17 July*
<u>Culex tritaenior.</u>	24 July	24 July	31 July	24 July	24 July
<u>Culex pipiens</u>	26 June	3 July*	26 June	19 June	24 July
Period of Operation	29 May to 15 Oct.	3 July to 15 Oct.	5 June to 8 Oct.	29 May to 15 Oct.	10 July to 30 Sept.

1. Data from Petrakis

* Based on very small numbers

* In our 1949 report (1) it was erroneously reported that the peak week for C. tritaeniorhynchus was the week of 24 July. This was true in terms of total number of the species collected, but was not true for the collection rate (number collected divided by number of collections). The peak week for Culex tritaeniorhynchus as determined by collection rate was the week of 31 July.

Table XIII. Mosquito Population Peaks in 1950 (Tokyo)

<u>Mosquito Species</u>	<u>Resting Stations</u>	<u>Bait Traps</u>	<u>Light Traps</u>	<u>Biting Collections</u>
<u>Anopheles hyrcanus</u>	9 July	16 July	9 July	30 July*
<u>Aedes vexans</u>	9 July	9 July	9 July	9 July*
<u>Culex tritaeniorhynchus</u>	30 July	23 July	23 July	23 July
<u>Culex pipiens</u>	25 June	9 July	23 July	23 July
Period of Operation	14 May to 30 Sept.	11 June to 30 Sept.	20 May to 30 Sept.	18 June to 30 Sept.

* Based on very small numbers

Explanations for the differences found in Tables XII and XIII are not limited to a few factors. Examination of the data from which some of the population peak dates were obtained reveals that several of the findings were based on collection of very small numbers of mosquitoes. Some of the discrepancies may also be attributed to operational difficulties encountered in the routine collection of mosquitoes. Prominent in this respect are the idiosyncrasies of local electrical current. The irregularity of this current is sometimes responsible for sizeable variation in trap catches. Operational difficulties are also encountered in animal bait trapping and in human biting collections. In the case of the former, animals will sometimes tear wire screening while in human biting surveys, the instructions for collection can be carried out with varying degrees of faithfulness. Weather is a third factor which is concerned here. Thus, adult resting stations are sometimes inaccessible during heavy rains, and light and bait traps are rendered inoperable. The elimination of a single station which would ordinarily produce a heavy yield of a given mosquito species might thereby result in an entirely disproportionate lowering of the collection rate for this species during that week. The picture is further obscured by inherent limitations in the various methods of collection. Two years' experience in Tokyo have indicated, for example, that resting station collection is probably the least reliable method for obtaining population trends of Culex tritaeniorhynchus. This species will only be found resting in numbers in animal shelters, and collections in other types of habitats are very unproductive. Sharper delineation of trends in this species can be obtained by animal bait trap or by light trap placed inside animal shelters. Not of the least importance in conducting controlled population studies is the attitude of collector personnel during very hot or rainy weather.

Based on the experience of the past two years' mosquito population surveys, the following observations might be made on the relative values of the various mosquito collection methods in epidemiological studies on Japanese B encephalitis.

Resting Station Collections - This method appears to be particularly valuable for the collection of data on Culex pipiens since large numbers of this species can be obtained by this method, while light traps and animal bait traps give poor yields of this species. Remarks in the previous paragraph indicate why this method is a poor one for obtaining data on C. tritaeniorhynchus populations. This collection method depends largely on the resting trophisms of mosquitoes and care must be exercised in setting up resting stations to select habitats from which data can be obtained on the mosquito species with which the investigation is concerned. With some species which prefer natural habitats to man-made habitats for resting, this is sometimes difficult or impossible to do. One important consideration in undertaking a resting station collection program, is that the time devoted to these collections can be markedly disproportionate to the volume of material collected.

Light trap Collections - Population changes of Culex tritaeniorhynchus, Anopheles hyrcanus, and of Aedes vexans can be detected quite readily through the use of strategically placed light traps. Culex pipiens is taken in smaller numbers and population curves obtained by this method are therefore less reliable for this species. In Tokyo,

operational difficulties limit the effectiveness of this method, as was particularly evident during 1949. However, when equipment is operated under close supervision, this method can produce very large volumes of material with relative ease and with a minimum of time and manpower. During 1950, with the improved location of light traps, the importance of trap locations became quite evident (Table V). As was pointed out in the previous discussion of light trap collections, differences existed not only in the size and the proportionate representation of species, but also in mosquito population curves. This finding necessitates setting up a program involving a number of traps and can easily result in trap yields so large as to completely overwhelm the mosquito identification facilities of the investigation. This problem can perhaps best be solved by making sample determinations and then applying a weight formula to the remainder of the catch. Caution must then be exercised that the sample obtained is a random sample, a sample of adequate size and that the catch from the trap is free from all extraneous material and insects.

Animal bait trap collection - This method is particularly sensitive to changes in the C. tritaeniorhynchus, Anopheles hyrcanus, and Aedes vexans populations. It does not yield sufficiently large numbers of Culex pipiens to provide accurate measurements on this species. Animal bait trap collections have the advantage of responding readily not only to changes in population size but also to the biting intensity of a mosquito population. While the method yields large numbers of mosquitoes, practical requirements limit the placement of these traps to a few locations, and considerable variation in mosquito bait trap catches will be found in traps baited with a given animal species when traps are placed in different localities (Table VIII). It has also been pointed out in the discussion of results obtained with animal bait traps that no single host animal can be used exclusively as a bait animal, since no single animal is sufficiently attractive to all mosquito species (Table VII). From data obtained thus far, the horse appears to be closest to the ideal animal bait in Japanese B encephalitis studies, but it should be noted that yields of Culex pipiens were very small with this animal.

Human Biting Collections - Population studies by human biting collection appear, at first glance, to be an ideal method for studying relationships between mosquito population and the course of epidemics. While the method does possess several advantages, it also is characterized by limitations. When conducted properly, this method offers direct evidence of the mosquito species attacking man and variations in the attack rate. It measures, not only differences in population size, but also variations in the biting intensity of species, which may be determined by factors other than population size, i.e. weather and light intensity. It has the disadvantages of being very time consuming, of producing rather small mosquito catches, and the results can vary markedly with the adeptness of the collector. Where collectors are used from personnel outside the investigative group, as was the case in the present survey, there is the additional problem of disinterest developing in the collectors as the season progresses. An attempt was made in the present study to forestall waning interest by the judicious use of commendations and publicity.

Larval Collections - A detailed discussion of the limitations of larval collection for mosquito population was given in 1949 (1). For the reasons enumerated in that report, no larval collection program was undertaken during 1950. In that discussion no mention was made of the larval check point system. This method of collection, which is frequently utilized in malaria studies, consists of the designation of a number of mosquito breeding situations as check points to be visited regularly at designated intervals. This collection method, like that of adult resting station collections, results frequently in an expenditure of time entirely disproportionate to the value of the results obtained, since large numbers of check points must be established for accurate evaluation of population changes. It has the further disadvantage that check points all too frequently will dry up in hot dry weather. This method has greater utility for the evaluation of mosquito control measures, than for the objectives of the present studies.

From the observations made during the past two years, it would appear that the most realistic approach to the problem of attempting correlation of mosquito population trends with the course of epidemics and epizootics of Japanese B encephalitis, would involve a combination of light trap collections, animal bait trap collections, and human biting collections. It should also be emphasized that none of the collection methods employed can provide anything but a crude measurement of mosquito populations. Despite all attempts at standardization, all traps, collection stations and other collection methods or devices may provide yields at variance with the actual mosquito population. It is therefore necessary to assemble data from various sources and to consider such data as a rough index of population trends.

RELATION OF WEATHER TO MOSQUITO POPULATIONS: Data on weather conditions during the period of these studies have been compiled and examined to determine if any weather factors bear a relationship to trends in mosquito populations. A graph illustrating mean weekly temperatures and mean weekly relative humidities as well as total weekly precipitation is provided in Figure 8. Examination of this graph with reference to mosquito population curves (Figures 1-7) fails to establish any well defined relationship between population trends in the several mosquito species concerned and in the three weather factors considered. These findings are consistent with those obtained in 1949 (1).

RELATION OF MOSQUITO POPULATIONS TO JAPANESE B ENCEPHALITIS IN 1950: One of the major objectives of the entomological program during the past two years has been to determine if any relationship exists between population curves of the various mosquito species and the course of epidemics and epizootics of Japanese B encephalitis. As pointed out previously, the methods of mosquito collection employed do not apply equally well to all species. For this reason it has been necessary to construct mosquito population curves on diversified bases. Data used for the 1950 epidemic of Japanese B encephalitis are based on reported cases by date of onset.

A comparison of the population curves of Anopheles hyrcanus and the 1950 Tokyo Japanese B encephalitis epidemic is presented in Figure 9. Results obtained by horse-baited traps and light traps were used since human biting collections of this species were small. The mosquito population curves are based on the average number of mosquitoes taken per trap-night during each week of the period studied. The epidemic curve illustrates the total number of reported human cases occurring in Tokyo each week. It will be noted that the population curves obtained by the two methods illustrated do not coincide. Thus, the period extending between the population peak and the epidemic peak represents four to five weeks. In general, the population curves obtained bear a resemblance to the epidemic curve, particularly in the case of the animal bait trap curve. Since information available suggests a 10-24 day incubation period in man (6), it appears that the spread between population peak and epidemic peak is too large to indicate a vector relationship.

A similar graph for Aedes vexans has been prepared in Figure 10. With this species, while the population curves obtained by the two collection methods show some variation, the two curves coincide with regard to the peak population week. Here the interval between population peak and epidemic peak is indicated as five weeks, considerably longer than the probable incubation period. These population curves again bear some resemblance to the epidemic curve.

Comparison of Culex pipiens population curves to the 1950 Tokyo epidemic is illustrated in Figure 11. Population curves are based on the number of mosquitoes biting per man-hour as determined in the human biting collection program, and on the mean number of mosquitoes taken in adult resting station collections. From this data, it can only be stated that the interval between the C. pipiens peak and the epidemic peak was three to seven weeks and the population curves vary markedly from the epidemic curve in general appearance.

In Figure 12 the relationship of the Culex tritaeniorhynchus population to the Tokyo epidemic is illustrated by curves obtained from human biting collections and from horse-baited traps. It will be noted that the C. tritaeniorhynchus biting curve shows marked similarity to the epidemic curve, but the correspondence of the curve obtained from animal bait trap collections to the curve of the epidemic is even more striking. The bait trap collection curve bears so strong a resemblance to the epidemic curve that the two can almost be superimposed upon one another. The interval between the population peak period, the week of 23 July and the epidemic peak period, the week of 13 August covers three weeks, which would roughly coincide with what is believed to be the incubation period of the disease. That the strong resemblance of the horse-bait curve is not purely fortuitous is indicated by the data obtained from traps baited with cows and those baited with pigs. Reference to Figure 3 will indicate population curves for C. tritaeniorhynchus which are markedly similar regardless of the animal used as bait.

Fig. 8 Weather During 1950 Mosquito Breeding Season

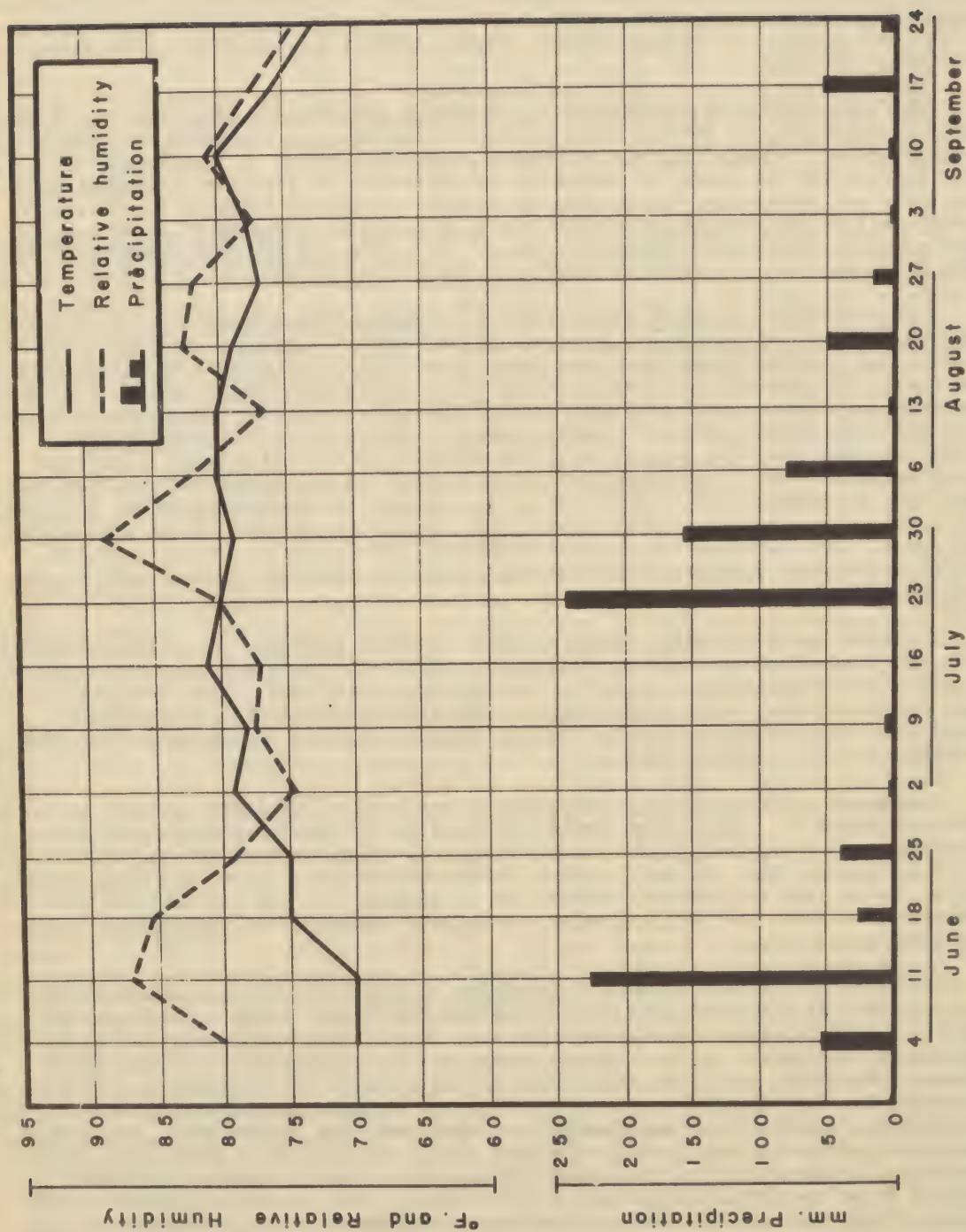


Fig. 9 Relation of Anopheles hyrcanus to Tokyo Encephalitis Epidemic of 1950

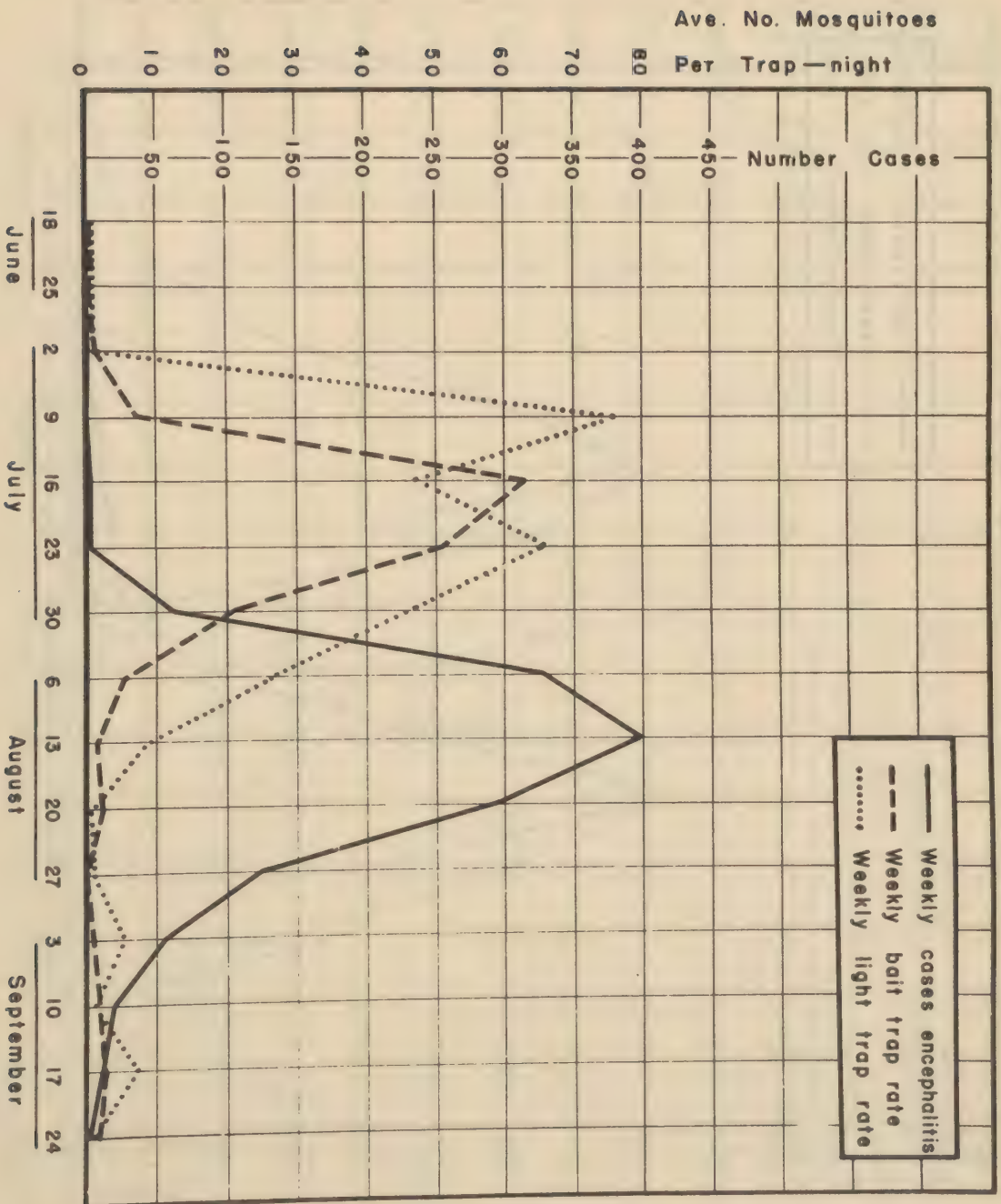


Fig. 10 Relation of *Aedes vexans* to Tokyo Encephalitis Epidemic of 1950

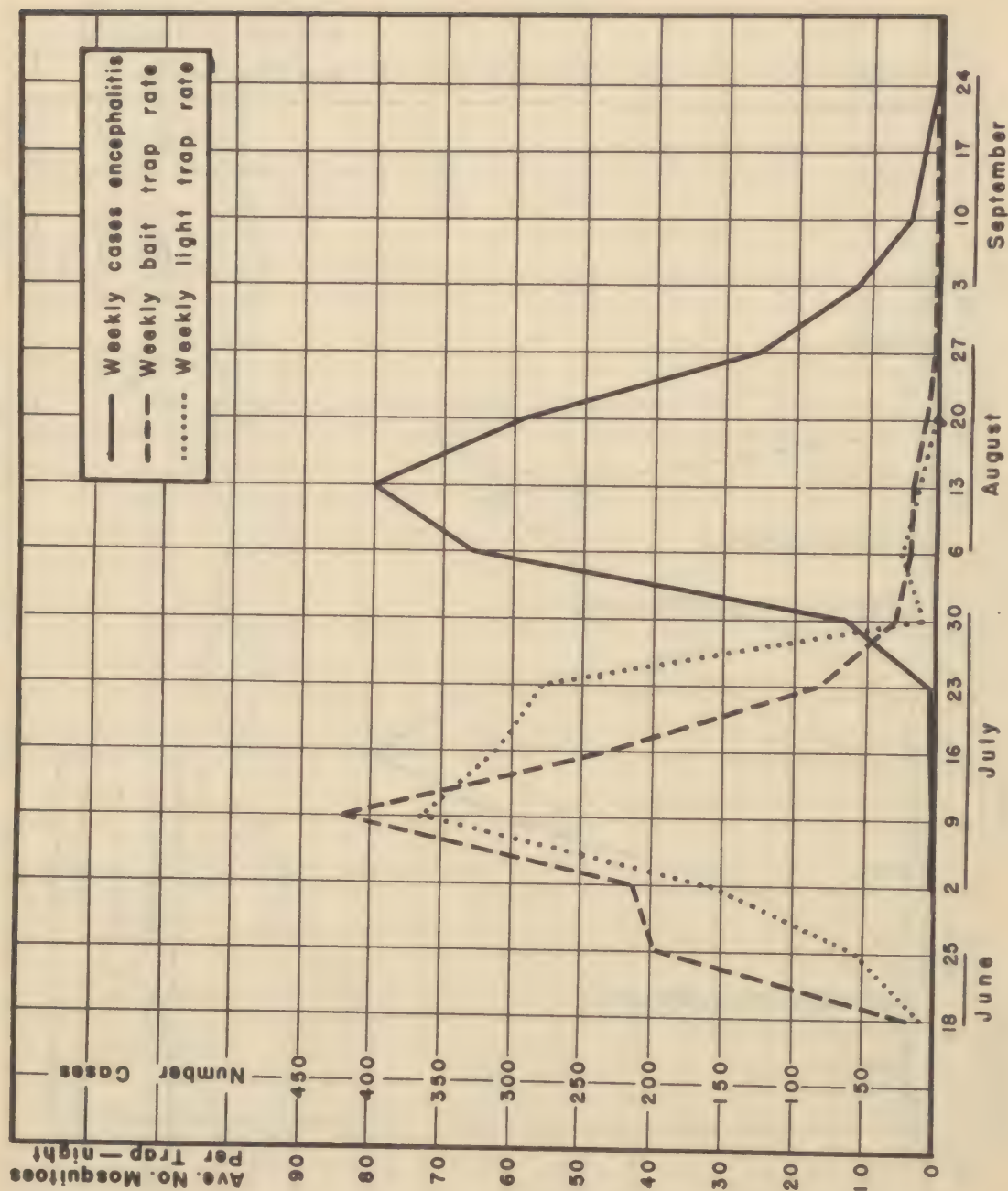


Fig. 11 Relation of Culex pipiens to Tokyo Encephalitis Epidemic of 1950

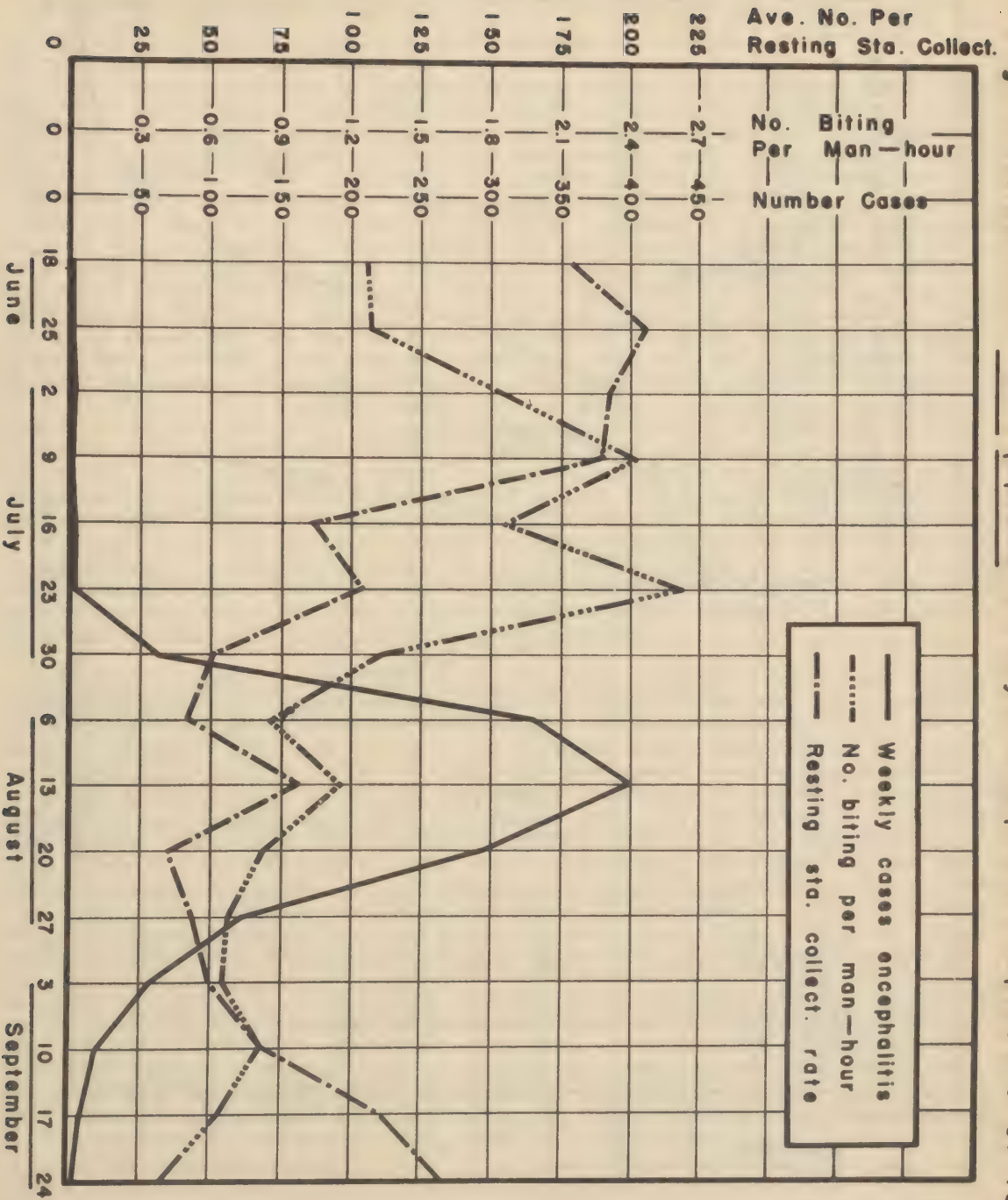
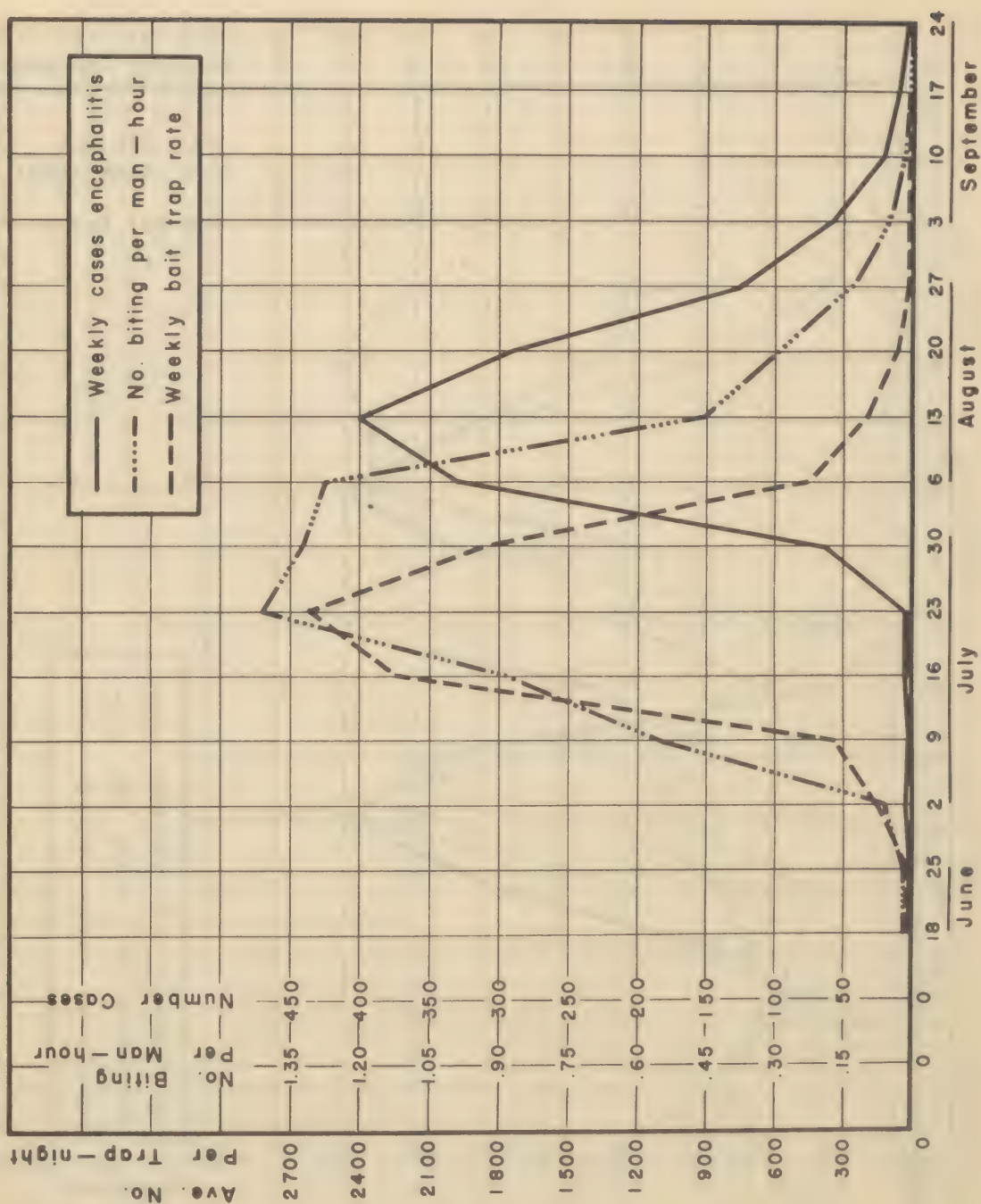


Fig. 12 Relation of *Culex tritaeniorhynchus* to Tokyo Encephalitis Epidemic of 1950

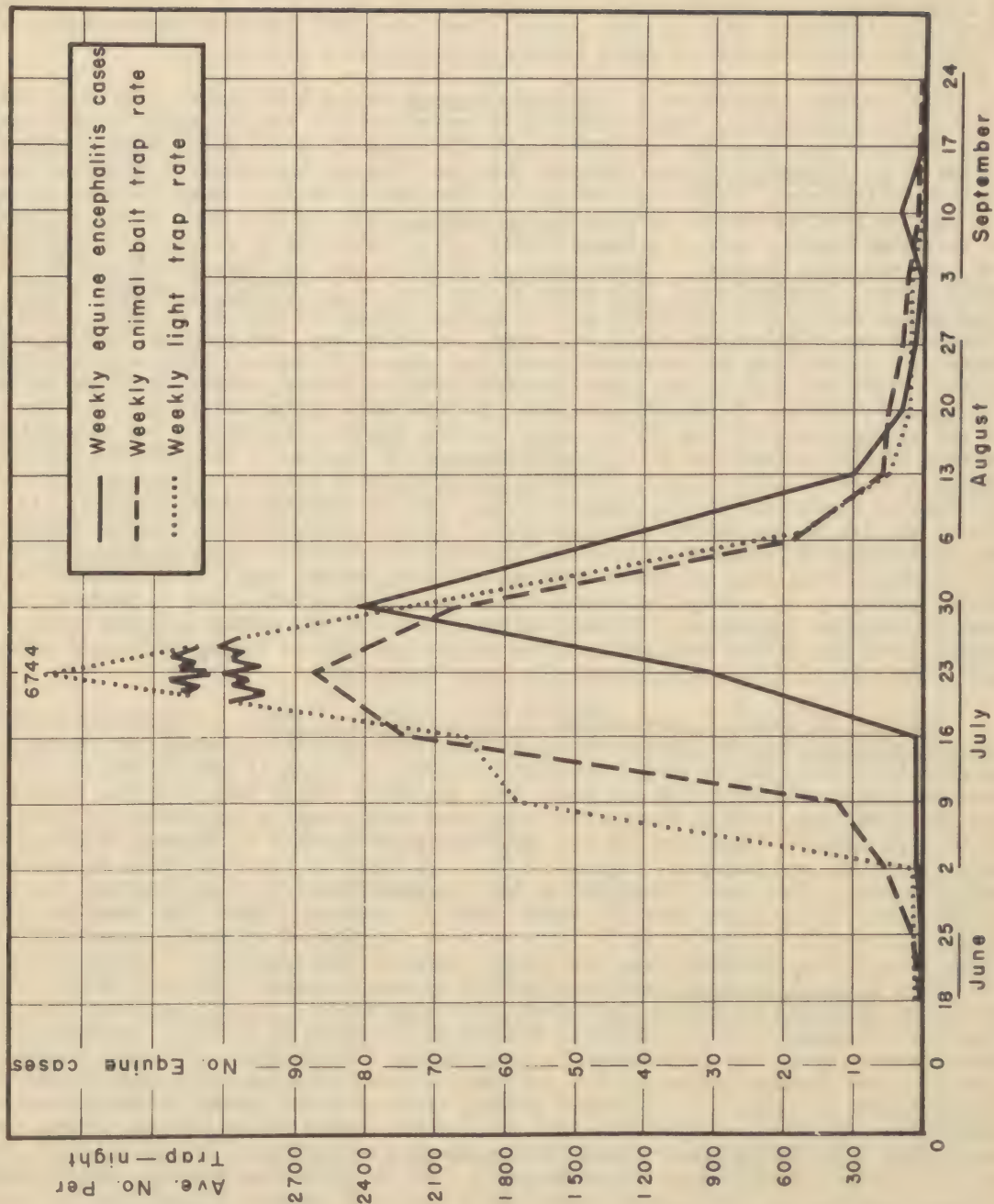


Essentially, the curve for the 1950 Tokyo Japanese B encephalitis epidemic is a normal distribution curve. All of the population curves of Culex tritaeniorhynchus show a similar pattern. Curves of Anopheles hyrcanus and Aedes vexans also tend toward a normal distribution, but they differ from Culex tritaeniorhynchus in one important respect. While the seasonal pattern of Culex tritaeniorhynchus falls within the estimated incubation period, the more extended interval between the epidemic and the curves of A. hyrcanus and A. vexans seems to eliminate the possibility of a causal relationship. As will be indicated later in this report, there are other and more important data supporting the incrimination of Culex tritaeniorhynchus as a vector.

The findings relative to C. tritaeniorhynchus during 1950 differ from those indicated for the 1949 survey. These differences necessitated a reexamination of the results from the previous year. In our 1949 report (1), comparison was drawn between population curves of C. tritaeniorhynchus obtained that year through horse-baited traps and resting station collections. These resulted in the observation that a 7 week interval extended between the peak population of C. tritaeniorhynchus and the peak week of the epidemic. In that same report, data on a human biting survey conducted by Petrakis in Yokohama during 1949 was also reported. Unfortunately, this data was not compared then with the epidemic curve. Examination of this human biting data on C. tritaeniorhynchus reveals a twin peaked curve, with the first peak occurring during the week of 24 July, which coincided with our findings by animal bait trap, by light trap, and by adult resting station. However, a second peak was observed during the week of 21 August, which was not apparent in the curves obtained by the three aforementioned collecting methods utilized in Tokyo. The interval between the second peak noted in the human biting collections and the peak of the epidemic in 1949 was three weeks. In this manner, correlation can be pointed out between population peaks of C. tritaeniorhynchus and Japanese B encephalitis epidemics during the years 1949 and 1950. However, the problem of evaluating the reliability of the various population curves obtained during 1949 in relation to the epidemic, is a difficult one due to the limited accentuation of careful evaluation of mosquito populations. The fundamental differences between the population curve of C. tritaeniorhynchus obtained by Petrakis during 1949 and those obtained by other methods might be an indication that biting intensity by a mosquito species can show marked variation due to factors independent of mosquito population. If such variation occurs, the marked parallel between the biting curve and curves obtained by other collection methods during 1950 would indicate that such variation does not necessarily occur every year.

RELATION OF MOSQUITO POPULATIONS TO JBE EPIZOOTIC IN 1950: A comparison of Culex tritaeniorhynchus population curves obtained in 1950 with the epizootic of Japanese B encephalitis occurring in horses in the Kanto area of Japan is made in Figure 13. Comparison of mosquito data with the Kanto area epizootic rather than with the Tokyo area was necessary, as in 1949, due to the small size of the equine population of Tokyo. The Kanto area includes Tokyo and the six surrounding prefectures of Ibaragi, Tochigi, Gunma, Saitama, Chiba, and Kanagawa. Epizootic data are based on reported cases by approximate date of onset. This data, furnished by the Japanese Ministry of Agriculture, was stated to be within two or three days of actual onset of symptoms. Since this data has been graphed in weekly intervals, it is believed that the plotted epizootic curve will, therefore, show little deviation from the actual course of the epizootic. Population curves for Culex tritaeniorhynchus have been plotted by weekly animal bait trap rates, using horses as bait, and by weekly light trap collection rates. It will be noted that the peak week obtained by both collection methods was the week of 23 July, one week prior to the epizootic peak. No information is available on the naturally occurring incubation period of the disease in horses, but information based on experimental infections has been obtained by Burns (7). Eighteen horses, three of which showed no neutralization titres for Japanese B encephalitis, and 15 of which showed neutralization titres were challenged with the Nakayama strain of Japanese B encephalitis. Dosage was 5 cc. of a $10^{-7.5}$ dilution administered intracerebrally. Of the three non-immunes, two developed typical symptoms in four days and died, while the third showed typical symptoms after 5 days and subsequently recovered. None of the immune animals developed symptoms, but all showed very marked rises in complement fixation and neutralization titres. If the natural incubation period roughly coincides with the 4 to 5 day incubation period noted in this experimental study, then the 1 week interval between the C. tritaeniorhynchus peak and the epizootic peak may be of considerable significance.

Fig. 13 Relation of Culex tritaeniorhynchus to 1950 Kanto Encephalitis Epizootic



In the 1948 epizootic in the Kanto area, the reported interval between the peak of C. tritaeniorhynchus population and the epizootic peak was again one week (1). In 1949, constructing the C. tritaeniorhynchus population curve by means of animal bait traps and by light traps, a five week interval between population peak and epizootic peak was found. However, as previously pointed out in the discussion on the relation of the C. tritaeniorhynchus population to the 1949 Tokyo epidemic, if the curve for the human biting rate obtained by Petrakis (5) in Yokohama during 1949 is compared to the epizootic curve, the interval between the epizootic peak and the secondary biting peak noted during the week of 21 August is one week. It should also be pointed out in this regard that a secondary rise also occurred during the week of 21 August in the curve which we had obtained for C. tritaeniorhynchus from horse-baited traps (1). This rise was on a small scale and had not been previously considered to be of any significance. No similar rise during the week of 21 August 1949 had been observed in light trap collections or in resting station collections.

It is of interest to note that during four years, 1936, 1948, 1949, and 1950, for which information is available on both epidemic and epizootics of Japanese B encephalitis, the interval between peaks of the two has consistently been two weeks and that the epizootic has always preceded the epidemic. The possible explanations are immediately apparent. The first, that this occurrence is an indication that epidemics stem directly from epizootics in horses, and the second, that the constant interval observed reflects differences in the incubation periods of the disease in man and horse, and that epizootics and epidemics spring from a common source.

OVERWINTERING MOSQUITO SURVEY 1949-1950: The possibility that mosquitoes harbor the virus of Japanese B encephalitis over the winter has resulted in numerous surveys for overwintering and hibernating mosquitoes. Since Culex tritaeniorhynchus has long been suspected of serving as a vector of this disease, interest was focused largely on the overwintering habits of this species. Despite intensive efforts by entomologists to discover the overwintering form of Culex tritaeniorhynchus, little can be offered on the subject other than conjecture (2), (8).

During the period November 1949 to February 1950, an intensive survey was conducted in Tokyo covering approximately one third of the city. Particular emphasis was placed on animal shelters since this is the only type of habitat in which C. tritaeniorhynchus rests in numbers during the summer. Search for larvae, eggs, and pupae was also made in a wide variety of breeding situations. However, since species of the genus Culex typically overwinter in the adult stage, emphasis was placed on adult collection.

During this survey, the search for mosquito adults was made in a number of type habitats as shown in Table XIV.

A tabulation of the results of this survey is given in Table XV. It will be noted that 96 per cent of all adults collected were Culex pipiens. Four hundred nineteen, or approximately 10 per cent of all the Culex pipiens taken, were males. All Culex hayashii taken were females. The sex ratio of these two species is at variance with results obtained by LaCasse and Yamaguti (8). No adult Culex tritaeniorhynchus were taken in this survey.

Collections of mosquito larvae totalled 32, from which 596 larval identifications were made. These were as follows:

<u>Culex pipiens</u>	4 collections, 342 specimens identified
<u>Aedes togoi</u>	18 collections, 199 specimens identified
<u>Aedes japonicus</u>	6 collections, 35 specimens identified
<u>Armigeres obturbans</u>	2 collections, 19 specimens identified
<u>Uranotaenia bimaculata</u>	1 collection, 1 specimen identified

Contrary to the findings of Sasa (2) who states that larvae of Culex pipiens and Aedes japonicus disappear by early December, these two species were found in the larval state in late January. No larva, eggs or pupae of Culex tritaeniorhynchus were found in this survey.

Table XIV. Places Examined For Overwintering Mosquitoes

<u>Type Habitat</u>	<u>No. of Examinations</u>
Horse stables and shelters	112
Pig pens	67
Caves and bomb shelters	55
Bldg. basements and foundations	48
Cattle barns and shelters	40
Houses, apt's and hotels	39
Chickenhouses	35
Sewer culverts and manholes	30
Sheds	24
Benjos (latrines)	14
Goat shelters	12
Subway stations	11
Tunnels	3 (approx. 1 $\frac{1}{4}$ miles)
Total	<u>490</u>

Table XV. Collections of Hibernating Adult Mosquitoes

<u>Habitat</u>	<u>No. Col- lections</u>	<u>Culex pipiens</u>	<u>Culex hayashii</u>	<u>Culex orientalis</u>	<u>Culex mimeticus</u>	<u>Totals</u>
Horse stables	3	15	0	0	0	15
Caves and bomb shelters	25	3,825	79	21	4	3,929
Bldg. basements	2	220	0	0	0	220
Chickenhouses	1	9	0	0	0	9
Sheds	1	35	0	0	0	35
Benjos	1	30	0	0	0	30
Tunnels	2	190	3	9	0	202
Totals	<u>35</u>	<u>4,324</u>	<u>82</u>	<u>30</u>	<u>4</u>	<u>4,440</u>

Results of virus isolation attempts on adult overwintering mosquitoes were completely negative.

PRECIPITIN TESTS ON MOSQUITO BLOOD MEALS: Introduction - One of the less commonly used methods for determining the host trophisms of mosquito species is the testing of blood smears obtained from engorged mosquitoes by the precipitin reaction. During the 1949 and 1950 mosquito surveys, 3,968 blood smears were prepared from engorged mosquitoes. An additional 1,032 engorged mosquitoes which had been preserved by dry ice freezing in sealed ampules were also tested by the precipitin reaction, bringing the entire series to 5,000. Surveys utilizing the precipitin reaction for determining the source of mosquito blood meals have been accomplished in a number of geographical areas (9), (10), (11), (12), (13), (14), but a large scale precipitin blood meal survey for Japan had not been previously attempted. Three papers (15), (16), (17) have appeared in the Japanese literature which have either involved small series or in which mixed antisera have been employed. The data presented herein are believed to be the first involving a large series of smears, where highly specific antisera were used. Techniques Employed: The precipitin reaction was first adapted for use in the determination of the host source of mosquito blood meals by Uhlenhuth et al. (18) in 1908. Rice and Barber (10) modified and simplified the techniques in conducting the first large scale survey where the reaction was used. Since then, other modifications in techniques have been published (19), (20), and the specificity of the test has been subjected to more rigid scrutiny (21), (22).

Blood smears were prepared by expressing the blood from the engorged mosquito abdomens onto filter paper with an applicator stick. After each smear, the blood soaked portion of the stick was cut off to prevent contamination of succeeding smears. Collection data were written on each filter paper and the filter papers were placed in a glass jar for storage. At the end of the season the jar was sealed with adhesive tape and paraffin and placed in a refrigerator. Smears prepared in this manner were kept for as long as 1½ years without apparent ill effects.

The most difficult problem in precipitin surveys is the preparation of highly specific antisera of high titre. The method of antisera production which gave the best results in this study is a modification of the Holstein method (20), details of which are given below.

Blood was drawn from five sources as follows: Man, horse, cow, pig and birds (chicken, turkey; Japanese Jungle Crow, Corvus coronoides hondoensis; and the Eastern Spot-billed Duck, Anas poecilorhyncha zonorhyncha). The serum was separated from each by clotting and centrifugation, and sufficient merthiolate added to make a 1:10,000 solution. For each type of antiserum desired, a series of 3 rabbits, each weighing 2 to 2½ kg. were used, and each given the following course of inoculations:

1st day - 10 cc. of 10 per cent dextrose in normal saline intraperitoneally, followed by 0.5 cc. of serum intravenously 2 hours later.

3rd day - 10 cc. of 10 per cent dextrose in normal saline intraperitoneally, followed by 1.0 cc. of serum intravenously 2 hours later.

5th day - 10 cc. of 10 per cent dextrose solution in normal saline intraperitoneally, followed by 1.5 cc. of serum intravenously 2 hours later.

12th and 15th days - rabbits bled from marginal ear vein and checked for titre against the homologous serum and for specificity. Using a 1:5 dilution of the antiserum, the minimum standard for acceptable antiserum was set at a strong reaction with a 1:4,000 dilution of the homologous serum. Weak reaction with heterologous sera at 1:1,000 was considered to constitute non-specificity.

Several antisera were obtained with very high titres (1:160,000) but such antisera tended to give cross reactions. By dilution of such antisera with normal saline, cross reactions could usually be eliminated, still maintaining the minimum standard titre of 1:4,000 with the homologous serum. It was found that when diluents containing glycerine were used it was more difficult to make readings. In the actual performance of these tests, no antisera were used with a titre below 1:8,000 or with cross reactions at 1:1,000.

After determining by trial bleeding that an acceptable antiserum was present, the rabbit was fasted overnight and then bled out aseptically from the heart the following day. After separation of the serum, merthiolate was added at 1:10,000 and the antiserum stored in rubber capped bottles at ordinary refrigeration temperature. Antiserum obtained from each rabbit were stored in separate stock bottles. Each month thereafter, antisera were checked for titre with the homologous serum and cross checked for specificity. This was found to be a very important control, since occasionally antisera would lose titre and develop cross reactions which apparently were due to bacterial contamination.

In performance of the test on blood smears, clean glassware and equipment were used throughout. Antigens were prepared by cutting the blood smears into numerous pieces and leaching them out in 1.0 cc. of normal saline solution overnight in the refrigerator. Failure to refrigerate resulted in gross bacterial contamination, making readings extremely difficult. Antigens from frozen mosquito material were prepared by macerating the abdomens in 1.0 cc. of normal saline solution and allowing the mixture

to stand at refrigeration temperature overnight. Each antigen was then tested against the five antisera, human, equine, bovine, avian, and porcine. For this purpose, special precipitin tubes two inches long and two mm. in inside diameter were used. Series of tubes were set up in small wooden racks and 0.05 cc. of each of the five antisera was placed in a tube using 1.0 cc. tuberculin syringes with 2 inch 25 gauge hypodermic needles. Then 0.05 cc. of antigen was carefully layered over each of the five tubes. Readings were taken at 15, 30, and 60 minutes. If readings were negative at 60 minutes additional readings were taken at 1½ and 2 hours. Double reactions were uncommon, but when they occurred, the first and strongest reaction was considered specific for tabulation purposes.

Results - The material used in the precipitin survey was gathered throughout the mosquito breeding seasons of 1949 and 1950. Twelve species collected from a wide variety of habitats were represented in this series. Results of the precipitin tests by type habitat are presented in Table XVI. It will be noted that mosquito material taken from dairies produced 94.3 per cent bovine reactions, material from horse stables yielded 86.6 per cent equine reactions, pig pen material provided 92.1 per cent porcine reactions, and chicken houses produced 97.6 per cent avian reactions. Inconsistent results were obtained from houses, where only 31.7 per cent of the smears tested yielded human reactions, while 63.2 per cent yielded avian reactions. However, in a large proportion of the houses from which material was collected, chickens were evident in or around the premises. Subway stations, in which mosquitoes do not have much opportunity for host selection, yielded 57.6 per cent human reactions and 23 per cent avian reactions. From these results it is apparent that the resting trophisms of mosquitoes are not always consistent with their feeding habits.

Table XVI. Summary of Precipitin Tests by Type Habitat

Type Habitat	Human		Avian		Bovine		Equine		Porcine		Negative		Totals	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Dairies	9	0.6	16	1.0	1414	94.3	5	0.3	5	0.3	49	3.5	1498	100
Horse stables	33	2.7	60	5.0	2	0.1	1033	86.6	1	0.1	66	5.5	1195	100
Houses	165	31.7	329	63.2	11	2.1	2	0.5	0	0.0	13	2.5	520	100
Caves and Bomb shelters	27	11.9	176	77.8	5	2.4	3	1.3	3	1.3	12	5.3	226	100
Sheds	15	8.2	139	76.7	0	0.0	1	0.5	9	5.3	17	9.3	181	100
Subway stations	15	57.6	6	23.0	0	0.0	1	3.8	0	0.0	4	15.6	26	100
Chicken houses	2	0.3	547	97.6	1	0.1	3	0.5	1	0.1	6	1.4	560	100
Pig pens	0	0.0	19	5.3	1	0.2	2	0.5	330	92.1	6	1.9	358	100
Benjos (latrines)	4	40.0	4	40.0	0	0.0	0	0.0	0	0.0	2	20.0	10	100
Animal bait traps	0	0.0	2	2.2	15	15.1	55	55.5	27	27.2	0	0.0	99	100
Misc. and mixed	64	19.5	185	56.3	11	3.3	7	2.1	35	10.7	25	8.1	327	100
Totals	324	6.6	1483	29.6	1460	29.2	1112	22.2	411	8.4	200	4.0	5000	100

A more detailed analysis of the results of the precipitin survey can be obtained by comparison of Table XVII with Table XVIII. Taking Culex tritaeniorhynchus as an example, it will be found that the number of bovine positive reactions closely approximates the number of smears taken from dairies. It is of interest to note that only ten engorged C. tritaeniorhynchus were found in houses and that only two produced positive human reactions. The small number of human positives obtained with C. tritaeniorhynchus might be interpreted as evidence that this species does not attack man, and hence, could not be involved in the transmission of Japanese B encephalitis. That such a conclusion would be incorrect is established by reference to the results obtained in the human biting survey conducted in 1950. Reference to Table X indicates that C. tritaeniorhynchus was the second most numerous species taken in the human biting collections. An examination of Figure 7 shows that during the week of 30 July, C. tritaeniorhynchus was taken in equal numbers with C. pipiens and that during the week of 6 August, one week before the peak of the epidemic, C. tritaeniorhynchus was the most prevalent species biting or attempting to bite man. The discrepancies noted in the results obtained by human biting collection and by precipitin test leave the implication that many Culex tritaeniorhynchus attempt to bite man, but that few succeed in obtaining blood meals. This implication finds support

Table XVII. Species Distribution by Type Habitat - Precipitin Test Series

Species	Dairies	Stables	Houses	Caves and Bomb Shelters	Sheds	Subways	Chicken Houses	Pig pens	Benjos	Bait traps	Misc. & Mixed	Totals
<u>A. hyrcanus</u>	261	172	1	3	0	0	0	90	0	2	17	546
<u>A. obturbans</u>	97	24	1	0	0	0	0	1	0	3	1	127
<u>A. vexans</u>	123	124	0	0	0	0	0	2	0	17	1	267
<u>A. togoi</u>	6	15	5	2	1	1	2	1	0	0	9	42
<u>A. japonicus</u>	0	0	0	0	0	0	0	0	0	7	0	7
<u>A. nipponicus</u>	0	0	0	0	0	0	0	0	0	2	0	2
<u>A. albopictus</u>	3	0	0	1	0	0	0	0	0	1	0	5
<u>C. tritaeniorhynchus</u>	686	717	10	2	2	1	20	132	0	38	22	1630
<u>C. pipiens</u>	316	142	502	218	175	24	538	132	10	29	275	2361
<u>C. bitaeniorhynchus</u>	6	1	0	0	2	0	0	0	0	0	1	10
<u>C. mimeticus</u>	0	0	0	0	1	0	0	0	0	0	1	2
<u>M. ochracea</u>	0	0	1	0	0	0	0	0	0	0	0	1
	1498	1195	520	226	181	26	560	358	10	99	327	5000

Table XVIII. Summary of Precipitin Tests by Species

Species	Human	Avian	Bovine	Equine	Porcine	Negative	Totals
<u>A. hyrcanus</u>	4	5	243	150	98	46	546
<u>A. obturbans</u>	1	1	95	27	3	0	127
<u>A. vexans</u>	0	1	121	131	5	9	267
<u>A. togoi</u>	19	1	7	9	2	4	42
<u>A. japonicus</u>	0	0	0	6	1	0	7
<u>A. nipponicus</u>	0	0	1	1	0	0	2
<u>A. albopictus</u>	1	0	2	1	0	1	5
<u>C. tritaeniorhynchus</u>	2	35	674	730	151	38	1630
<u>C. pipiens</u>	307	1435	311	56	151	101	2361
<u>C. bitaeniorhynchus</u>	0	2	6	1	0	1	10
<u>C. mimeticus</u>	0	2	0	0	0	0	2
<u>M. ochracea</u>	0	1	0	0	0	0	1
Totals	334	1483	1460	1112	411	200	5000

in the observations made in 1949 (1) that only 29 per cent of the female C. tritaeniorhynchus collected in houses were engorged with blood, while 55%, 75%, and 83% of the females taken in horse stables, dairies and pig pens, respectively, by resting station collections were blood engorged. Sasa and Sabin (4) reported similar results in studies on Culex tritaeniorhynchus in Okayama. They found that this species was taken more frequently in the act of biting humans than any other mosquito species, but in animal bait trap studies, the percentage of females of this species which engorged on human blood was markedly lower than that of pig, dog, rabbit, goat, canary, and chicken. On the basis of their findings, they hypothesized that man was not the source from which Culex tritaeniorhynchus obtained the virus of Japanese B encephalitis. It is apparent that further observation and experimentation on the feeding habits of this species in relation to man is highly desirable. It is also noteworthy that while only 20 blood smears were obtained from C. tritaeniorhynchus collected in chicken houses, 35 avian positive reactions were obtained. Thus, while birds do not apparently constitute a major host group, the extent of feeding on birds would appear to be somewhat larger than is apparent from the collection of this species at rest in avian habitats. However, in 1949 (1) only 29% of the total number of C. tritaeniorhynchus taken at rest in chicken houses were found to be engorged, and Sasa and Sabin (4) report that only 11% of this species taken in chicken baited

traps were engorged.

Comparison of data on Culex pipiens in Table XVII and XVIII indicates close correlation between the smears taken in dairies and bovine positive reactions. The number of equine positive reactions was considerably smaller than the number of smears taken in horse stables. It was from this group that the largest number of negative precipitin reactions were obtained. Culex pipiens provided the largest number of human positive reactions, but it should be noted that these reactions accounted for only 60% of all the C. pipiens blood smears taken in houses. The remaining 40% of the blood smears produced avian positives. The probable explanation of these findings, as indicated earlier, was the co-habitation of chickens and man in a large number of the houses from which material was collected.

In general, the precipitin test results obtained with Anopheles hyrcanus, Armigeres obturbans, and Aedes vexans are proportionate to the size of the series obtained from the various types of animal shelters. Aedes togoi showed interesting results in that the largest proportion of human positive reactions 45% were obtained from this species. LaCasse and Yamaguti (8) state with reference to this species, "All our observations tend to indicate that the probable importance of this species as a potential vector has been highly overrated. This is particularly true in Japan with regard to the potential role which it may play in the transmission of Japanese B encephalitis. The literature repeatedly points with suspicion to A. togoi as a probable vector species. The conclusion that this mosquito is relatively unimportant is based upon extensive day and night biting collections over a four year period. It was taken only once in biting collections throughout the entire series of collections." In the same publication, LaCasse and Yamaguti state that Aedes vexans is a species of probable importance in the transmission of Japanese B encephalitis.

By reference to Table XVII and XVIII, it will be noted that while A. togoi yielded six human positive reactions from a series of 42 blood smears, Aedes vexans produced no human positives out of a much larger blood smear series of 267. It should also be noted that both species were taken in equal numbers in the human biting survey. From the data obtained during 1950 in Tokyo, it would now appear that A. togoi and A. vexans should be viewed with equal suspicion as possible vectors of Japanese B encephalitis.

In a series of blood smears obtained from mosquitoes collected in resting stations, distribution of the material by species will usually follow closely upon the relative proportions of the species collected in these stations. Reference to Table XIX will show that the blood smear material run in this precipitin test survey was distributed in the same general proportions as species collected by resting station during 1949 and 1950. This can easily result in a disproportionate representation of blood smear by species, since any species which tends to rest in foliage or vegetation would be poorly represented.

Table XIX provides other comparisons of interest. It will be noted that the ranking of mosquito species by positive human reactions in the precipitin test series differs considerably from results obtained by human biting collection. Aedes togoi which was represented in the blood smear series by only 42 specimens showed the highest percentage of human positive reactions. However, the low rank this species shows in attacking man in the human biting survey, throws a question on the significance of the findings by precipitin test. The importance of Aedes albopictus as a species attacking man is questionable since data on this species is limited to precipitin tests on only five specimens. However, this species ranked as the third most important species in the human biting survey. The strong affinity of Culex pipiens for man is established by first rank in the human biting survey and by third rank in the precipitin test survey. Table XVIII shows that C. pipiens provided the largest series of human positives in the precipitin survey, while the first and second ranking species provided human positives from much smaller series. It should also be noted in Table XVIII that while C. pipiens showed a strong affinity for man, a much stronger preference for avian blood was evident.

Table XIX. Comparison of Potential Mosquito Vectors

No. Collected from Resting Stations 1949 and 1950	Size of Blood Smear Series	Positive Human Reactions in Precipitin Tests	No. Collected in Human Biting Survey
<u>C. pipiens</u> (102,723)	<u>C. pipiens</u> (2,361)	<u>A. togoi</u> (45%)	<u>C. pipiens</u> (3,186)
<u>C. tritaen.</u> (26,886)	<u>C. tritaen.</u> (1,630)	<u>A. albopictus</u> (20%)	<u>C. tritaen.</u> (1,115)
<u>A. hyrcanus</u> (3,680)	<u>A. hyrcanus</u> (546)	<u>C. pipiens</u> (13%)	<u>A. albopictus</u> (256)
<u>A. vexans</u> (1,024)	<u>A. vexans</u> (267)	<u>A. obturbans</u> (0.7%)	<u>A. obturbans</u> (221)
<u>A. togoi</u> (868)	<u>A. obturbans</u> (127)	<u>A. hyrcanus</u> (0.7%)	<u>A. hyrcanus</u> (52)
<u>A. obturbans</u> (609)	<u>A. togoi</u> (42)	<u>C. tritaen.</u> (0.1%)	<u>A. togoi</u> (32)
<u>(subalbatug)</u>			
<u>A. albopictus</u> (305)	<u>C. bitaen.</u> (10)	-----	<u>A. vexans</u> (32)

There is a strong possibility that variations in results obtained by precipitin test survey and by human biting collection may occur due to the reluctance or inability of a species to engorge on the blood of a given host species after attacking this host. As pointed out earlier, this may occur in the case of C. tritaeniorhynchus attacking man.

VIRUS ISOLATION STUDIES ON MOSQUITOES COLLECTED IN 1949: During 1949, mosquito collections were undertaken by the Department of Entomology in Tokyo with the objective of obtaining large volumes of mosquito material for attempts at isolating the virus of Japanese B encephalitis. This survey was presented in detail in the 1949 Annual Report of this laboratory. Studies were conducted jointly with the Department of Virus and Rickettsial Diseases.

Additional mosquito collections were made in Okayama Prefecture by the 207th Malaria Survey Detachment, during the period of 25 July to 26 August and 9 September to 15 September. A total number of 219 collections were made from 31 adult resting stations. All resting stations were animal shelters, housing horses, cows, pigs, sheep, or chickens. A summary of all mosquito material which was preserved from the Tokyo and Okayama collections and subsequently tested for Japanese B encephalitis virus at the 406th Medical General Laboratory is presented in Table XX. In addition to the material tested for the virus of Japanese B encephalitis at this laboratory, shipments of frozen mosquitoes which had been collected in Tokyo during 1949, were sent to Dr. W. McD. Hammon, Hooper Foundation, University of California, where virus isolation tests were also undertaken. The volume and species distribution of the material sent to the Hooper Foundation closely parallels the list of material from Tokyo shown in Table XX.

The procedures used at the 406th Medical General Laboratory in virus isolation tests on the mosquito material collected in Tokyo and Okayama during 1949 were as follows:

1. All mosquito identifications were rechecked before processing of mosquitoes.
2. Where tubes contained 85 to 100 mosquitoes, they were run as separate lots. Tubes containing lesser amounts of mosquitoes were pooled to make a total of one hundred, using only tubes containing a single mosquito species, which had been collected during a period no greater than a week.
3. Mosquitoes were emulsified in Ten Broeck grinders, using a Sorensen buffer of pH 8.2 containing 750 units of penicillin per cc. and streptomycin at a strength of 250 micrograms of activity. Four cc. of the buffer-antibiotic solution were used per 100 mosquitoes.
4. Six mice were inoculated for each lot of mosquitoes, using 0.03 cc. of the mosquito suspension intracerebrally. Mice were two to three weeks old at time of inoculation.

Table XI. 1949 Mosquito Material tested for Japanese B Encephalitis Virus

<u>Species</u>	<u>Tokyo</u>	<u>Okayama</u>	<u>Totals</u>
<u>Anopheles hyrcanus</u>	1,208	8,817	10,025
<u>Armigeres obturbans</u>	449	6,023	6,472
<u>Aedes japonicus</u>	7	0	7
<u>Aedes togoi</u>	402	0	402
<u>Aedes nipponicus</u>	4	0	4
<u>Aedes albopictus</u>	179	0	179
<u>Aedes vexans</u>	1,055	0	1,055
<u>Culex hayashii</u>	67	0	67
<u>Culex bitaen.</u>	18	14	32
<u>Culex tritaen.</u>	11,371	23,950	35,321
<u>Culex orientalis</u>	1	0	1
<u>Culex pipiens</u>	17,008	314	17,322
<u>U. bimaculata</u>	8	0	8
 Totals	 31,777	 39,118	 70,895

5. Two of the workers handled no virus throughout the course of this study. A third worker who handled virus at intervals during the project, did no work on this project on days when he was handling virus.

From the entire series of material run, two isolations were obtained, both from collections of Culex tritaeniorhynchus females. Both isolations came from material collected in midsummer in Okayama. Identifications of the isolates as Japanese B encephalitis virus was accomplished by cross-neutralization and by cross-protection tests.

Hammon (23) reports one isolation from 100 Culex tritaeniorhynchus collected on 13 August 1949 from Ueno Park in Tokyo. This virus was tentatively identified as Japanese B encephalitis by cross-complement fixation test. Isolation was effected directly from the mosquito suspension by inoculation of both baby and three week old mice. Hammon is of the opinion that the small number of isolations obtained resulted from inadequate refrigeration of specimens during shipment.

Included in the mosquito material from Tokyo listed in Table XI were 2,114 Culex pipiens, 55 Culex hayashii and one Culex orientalis female adults which were collected in the 1949-1950 overwintering survey. All of this material was tested for virus isolation at the 406th Medical General Laboratory. These mosquitoes were run through four blind passages in mice, in addition to the initial inoculation. Results with this material were completely negative.

VIRUS ISOLATION STUDIES ON MOSQUITOES COLLECTED IN 1950: Introduction - The isolation of Japanese B encephalitis virus from three species of Japanese mosquitoes, Culex tritaeniorhynchus, Culex pipiens, and Anopheles hyrcanus sinensis caught in nature has been reported in numerous papers (23-36). Russian workers have also reported isolations of this virus from C. tritaeniorhynchus and C. pipiens taken in nature in the Far Eastern maritime provinces of the U.S.S.R. (37), (38). The validity of some of these reports of virus isolation has been questioned (35), (39), (40), (41), (42) due to reported spontaneous infection of mice raised in endemic areas, and due to the possibility of laboratory contamination, particularly where blind passage has been necessary. This study has yielded a very large series of isolates and although this work was undertaken in an endemic area, it is believed that the results themselves will largely answer these questions bearing on the validity of the isolations.

Material Tested - Mosquito material collected during 1950 has been tested for the presence of Japanese B encephalitis virus both by this department and by the

Virus and Rickettsial Department. During the mosquito breeding season of 1950, a total of 2,010 tubes of mosquitoes were frozen and preserved for subsequent virus isolation attempts. These tubes contained a total of 158,817 female adult mosquitoes. A breakdown of this material by species is given in Table XXI. As will be noted from the Table, less than half of the material preserved from the 1950 collections has been tested to date. At the time this report is being written, testing of mosquito material is still being conducted, and this report must therefore be considered incomplete.

Table XXI. Summary of 1950 Mosquito Material Preserved and Tested for Virus of Japanese B Encephalitis

Species	Frozen Material				Fresh Material			
	No. tubes Prepared	No. mos- quitoes Tubed	No. tubes used by Virus Dept.	No. mos- quitoes tested by Virus Dept.	No. tubes used by Entomology Dept.	No. mos- quitoes tested by Entomology Dept.	No. lots used by Virus Dept.	No. mos- quitoes tested by Virus Dept.
<u>Anopheles hyrcanus</u>	85	3,991	0	0	32	1241	3	300
<u>Armigeres obturbans</u>	55	1,714	1	11	31	951	18	1369
<u>Aedes vexans</u>	89	4,234	3	79	38	1710	7	511
<u>Culex tritaen.</u>	1394	128,007	141	12,703	267	22,514	61	5963
<u>Culex pipiens</u>	383	20,825	86	5,381	116	6,073	16	1280
<u>Culex bitaen.</u>	2	21	0	0	2	21	0	0
<u>Aedes togoi</u>	2	25	0	0	2	25	0	0
Totals	2010	158,817	231	18,174	488	32,535	105	9423

Techniques - The techniques used for virus isolation from mosquito material was identical in both departments engaged in this work and are outlined as follows:

1. Mosquitoes were brought in from the field, anesthetized, identified, sealed in glass tubes, frozen on dry ice and stored in a dry ice reefer. All identification, tubing, and freezing was done in a room in which no virus work had been done previously. Identifications were made by professionally trained entomologists, all of whom had considerable experience in mosquito identification, specifically including local species. Prior to processing for virus isolations, all tubes of mosquitoes were rechecked for accuracy of identification.

2. Precautions against contamination included the washing of all tables, working surfaces, equipment, etc. prior to and at the completion of work on any lot of mosquitoes or of mice. Hands were washed in a similar manner. These precautions were rigidly enforced throughout the project.

3. Mosquitoes were ground in Ten Broeck grinders using a Sorensen buffer (pH 8.2) antibiotic mixture as the diluent. Each cubic centimeter of buffer contained 1,000 units of penicillin and 500 streptomycin micrograms of activity. Lots of 100 mosquitoes were ground in 4.0 cc. of the antibiotic solution, smaller mosquito lots receiving correspondingly smaller amounts of the antibiotic solution. Mosquito emulsions were permitted to stand for three hours at ordinary refrigeration temperature before inoculation into mice.

4. Mosquito emulsions were checked for sterility by inoculation of tryptose-phosphate broth and, in later phases of the work, also by the preparation of blood agar plates.

5. Each tube of mosquito emulsion was inoculated into five mice, 16-21 days old, each mouse getting 0.03 cc. intracerebrally and 0.1 cc. intraperitoneally. Mice were checked twice daily for symptoms and mortality readings. Emulsions of mouse brain were prepared in a manner similar to that used for mosquito emulsions, using 3.0 cc. of the antibiotic solution per brain. Passages were made by inoculating 10 mice. Four passages were made in each isolation, the last (fourth) passage going into 20 mice for the preparation of an antigen. A large proportion of the material tested by the Virus Department group was run through one blind passage, and a small series of tubes were also given one blind passage in the work of the Entomology group. However, only one isolate in the series reported was involved in blind passage material.

6. Antigens for complement fixation were prepared by a slight modification of the Casals (43) technique.

Results - From 488 tubes of mosquitoes tested to date by this department, 37 virus isolations have been obtained. The Virus and Rickettsial Department has also obtained a total of 38 virus isolations from 231 tubes of frozen mosquitoes and from 105 lots of fresh mosquitoes. All isolations obtained have been from Culex tritaeniorhynchus, while all other species have produced entirely negative results. From Table XXI it can be determined that the combined 75 virus isolates were obtained from a total of 41,180 C. tritaeniorhynchus tested, while the other species, represented by a total of 18,952 mosquitoes yielded negative results. A summary of all virus isolations obtained to date by both departments is presented in Table XXII. All isolations were obtained from mosquitoes taken from animal bait traps or from animal shelters. Reference to Table XXII will show the following distribution:

Horse-baited traps	35 isolations
Cow-baited traps	20 isolations
Pig-baited traps	14 isolations
Dairies	4 isolations
Stables	1 isolation
Pig pens	1 isolation
Total	75 isolations

The earliest date of collection from which an isolation was obtained was 5 July, while the latest collection date yielding an isolate was 30 August.

Of the 37 virus isolates obtained by the Department of Entomology, to date 36 have been identified as Japanese B encephalitis by titres of 1:8 to 1:64 in the complement fixation test, while all 38 isolates obtained by the Virus and Rickettsial Department have shown titres of 1:8 to 1:256 for Japanese B encephalitis in complement fixation tests. All isolates have shown the same symptomological pattern and have produced identical mortality pictures in mice. All complement fixation tests have been conducted by the Virus and Rickettsial Department and have included sera of Japanese B encephalitis, St. Louis encephalitis, and Eastern Equine encephalitis or Western Equine encephalitis.

Table XXII. Summary of Virus Isolations from Mosquitoes (Culex tritaeniorhynchus Collected in Tokyo During 1950

<u>Test No.</u>	<u>Tube No.</u>	<u>Coll. No.</u>	<u>Date Coll.</u>	<u>Collection Locality</u>	<u>Habitat or Bait</u>	<u>No. Mosq.</u>	<u>Isolation By</u>
E-32	156	319	5 July	Ueno Park B.T.	Cow	100	Entomology
E-114	212	377	11 July	Ikenoue	Dairy	78	Entomology
FM-72	242	397	13 July	Jikeikai B.T.	Pigs	100	Virus
E-297	242	397	13 July	Jikeikai B.T.	Pigs	100	Entomology
M-25	---	437	18 July	Ueno Park B.T.	Horse	100	Virus
M-27	---	439	18 July	Hatagaya B.T.	Cow	100	Virus
E-99	318	439	18 July	Hatagaya B.T.	Cow	100	Entomology
E-372	364	468	21 July	Yoyogi B.T.	Pigs	100	Entomology
FM-76	369	477	22 July	Hatagaya B.T.	Cow	100	Virus
FM-87	369	477	22 July	Hatagaya B.T.	Cow	100	Virus
E-190	380	486	24 July	Yoyogi	Stable	100	Entomology
E-203	383	487	24 July	Yoyogi B.T.	Pigs	100	Entomology
E-344	383	487	24 July	Yoyogi B.T.	Pigs	100	Entomology
E-348	383	487	24 July	Yoyogi B.T.	Pigs	100	Entomology
E-366	383	487	24 July	Yoyogi B.T.	Pigs	100	Entomology
E-188	404	506	26 July	Yoyogi B.T.	Pigs	100	Entomology
E-339	404	506	26 July	Yoyogi B.T.	Pigs	108	Entomology
M-44	---	510	26 July	Yoyogi B.T.	Horse	100	Virus
E-192	408	510	26 July	Yoyogi B.T.	Horse	77	Entomology
E-347	408	510	26 July	Yoyogi B.T.	Horse	100	Entomology
E-388	408	510	26 July	Yoyogi B.T.	Horse	100	Entomology
FM-44	419	519	27 July	Hatagaya B.T.	Cow	100	Virus
M-45	---	519	27 July	Hatagaya B.T.	Cow	100	Virus
FM-12	432	530	28 July	Yoyogi B.T.	Horse	100	Virus
FM-55	432	530	28 July	Yoyogi B.T.	Horse	100	Virus
FM-63	432	530	28 July	Yoyogi B.T.	Horse	100	Virus
E-333	432	530	28 July	Yoyogi B.T.	Horse	100	Entomology
E-337	432	530	28 July	Yoyogi B.T.	Horse	100	Entomology
E-246	439	534	28 July	Shimotakaido	Dairy	100	Entomology
E-399	442	536	28 July	Yoyogi B.T.	Pigs	100	Entomology
E-393	449	544	31 July	Yoyogi B.T.	Horse	100	Entomology
E-281	453	545	31 July	Yoyogi B.T.	Pigs	12	Entomology
M-57	---	562	2 Aug.	Ueno Park B.T.	Pigs	100	Virus
E-320	472	562	2 Aug.	Ueno Park B.T.	Pigs	100	Entomology
E-356	476	563	2 Aug.	Yoyogi B.T.	Horse	100	Entomology
M-61	---	571	3 Aug.	Ueno Park B.T.	Horse	100	Virus
E-324	483	571	3 Aug.	Ueno Park B.T.	Horse	100	Entomology
E-343	483	571	3 Aug.	Ueno Park B.T.	Horse	100	Entomology
M-62	---	572	3 Aug.	Hatagaya B.T.	Cow	100	Virus
E-314	485	572	3 Aug.	Hatagaya B.T.	Cow	100	Entomology
E-317	485	572	3 Aug.	Hatagaya B.T.	Cow	100	Entomology
M-64	---	576	3 Aug.	Horinouchi	Dairy	100	Virus
M-65	---	580	4 Aug.	Yoyogi B.T.	Horse	100	Virus
FM-54	497	580	4 Aug.	Yoyogi B.T.	Horse	100	Virus
E-335	497	580	4 Aug.	Yoyogi B.T.	Horse	100	Entomology
E-352	497	580	4 Aug.	Yoyogi B.T.	Horse	100	Entomology
E-354	497	580	4 Aug.	Yoyogi B.T.	Horse	100	Entomology
FM-99	510	593	7 Aug.	Tokyo Hort. School	Pig pens	100	Virus
FM-41	513	594	7 Aug.	Yoyogi B.T.	Horse	100	Virus
E-308	513	594	7 Aug.	Yoyogi B.T.	Horse	100	Entomology
E-309	513	594	7 Aug.	Yoyogi B.T.	Horse	100	Entomology
FM-5	512	595	7 Aug.	Ueno Park B.T.	Cow	100	Virus
M-68	---	604	8 Aug.	Hatagaya B.T.	Cow	100	Virus
FM-51	519	604	8 Aug.	Hatagaya B.T.	Cow	100	Virus
FM-73	519	604	8 Aug.	Hatagaya B.T.	Cow	100	Virus
E-311	519	604	8 Aug.	Hatagaya B.T.	Cow	100	Entomology

Table XXII. Continued

Test No.	Tube No.	Coll. No.	Date Coll.	Collection Locality	Habitat or Bait	No. Mosq.	Isolation By
M-69	---	605	8 Aug.	Ueno Park B.T.	Horse	100	Virus
M-70	---	606	8 Aug.	Ueno Park B.T.	Cow	100	Virus
E-321	521	606	8 Aug.	Ueno Park B.T.	Cow	79	Entomology
FM-94	529	614	9 Aug.	Yoyogi B.T.	Horse	100	Virus
E-400	529	614	9 Aug.	Yoyogi B.T.	Horse	100	Entomology
E-413	529	614	9 Aug.	Yoyogi B.T.	Horse	100	Entomology
FM-9	537	626	10 Aug.	Hatagaya B.T.	Cow	100	Virus
E-414	543	633	11 Aug.	Yoyogi B.T.	Horse	100	Entomology
FM-38	545	637	11 Aug.	Hatagaya B.T.	Cow	100	Virus
FM-31	553	645	14 Aug.	Yoyogi B.T.	Horse	100	Virus
FM-33	561	655	15 Aug.	Ueno Park B.T.	Horse	100	Virus
FM-105	568	663	16 Aug.	Yoyogi B.T.	Horse	100	Virus
FM-109	571	665	16 Aug.	Ueno Park B.T.	Pigs	100	Virus
FM-117	576	676	17 Aug.	Ueno Park B.T.	Horse	100	Virus
FM-115	578	677	17 Aug.	Hatagaya B.T.	Cow	100	Virus
FM-121	583	685	18 Aug.	Shimotakaido	Dairy	100	Virus
FM-116	582	687	18 Aug.	Yoyogi B.T.	Horse	100	Virus
M-85	---	770	30 Aug.	Yoyogi B.T.	Horse	100	Virus

Of the 75 isolates obtained to date, 69 have been recovered from mosquitoes collected in animal bait traps. The question immediately arises as to whether these mosquitoes obtained the virus from the bait animals or from other sources in nature. The answer to this question lies in an examination of the chronology of isolations obtained from mosquitoes collected in two bait traps, one the Yoyogi bait trap which was horse-baited; the other the Hatagaya bait trap which was cow-baited. In both traps, the same animal was used throughout the season as bait. By reference to Table XXIII, it will be found that the isolations obtained from material collected in both traps shows a dispersion over a collection period of one month or more. This dispersion period is considerably longer than the observed period of viremia in experimentally infected animals, and it would therefore follow that many, and possibly all, of the isolates obtained were acquired by the mosquitoes from sources other than the bait animals. It is of interest to note that neither of the bait animals showed symptoms at any time during the period of this study, despite the fact that the horse used in the Yoyogi trap was a non-immune animal. There is other evidence in Table XXII which suggests the unlikelihood of mosquitoes having obtained virus from bait animals while confined to the trap. It will be noted that an isolate was obtained from mosquitoes collected on the same date in a pig-baited trap at Yoyogi. The trap was located within 50 feet of the stable. On three other dates, 26 July, 28 July, and 31 July, mosquito collections taken from horse-baited and pig-baited traps at Yoyogi yielded virus isolates from both sources of collection. The two traps were located within 50 feet of one another. Similarly, at Ueno Park two collections of mosquitoes taken from horse-baited and cow-baited trap on 8 August yielded isolates. Again the two traps were located in close proximity. No emphasis has been placed, in testing mosquito material, on running paired lots to determine if this association of isolates existed. Thus, in the chance running of mosquito material to date, five instances have occurred within a collection period of 16 days where two sources of mosquito collections in a given locality have yielded virus isolates. This strongly suggests that mosquitoes in these localities were infected prior to entering bait traps.

In the analysis of the virus isolation data obtained from mosquitoes, an attempt has been made to compare the progression of mosquito infectivity with mosquito population trends and with the course of the epizootic and epidemic. In Table XXIV a tabulation has been prepared in weekly collection date intervals of all virus isolates obtained and of the number of tubes tested during each of these weekly intervals. From this information, percentage isolation has been calculated. Using the Culex tritaeniorhynchus population curve obtained from horse-baited traps, the weekly mean mosquito catches

Table XXIII. Chronology of Virus Isolates from Mosquitoes Collected in Two Bait Traps

Yoyogi Trap (Horse bait)		Hatagaya Trap (Cow bait)	
Date	No. Isolates	Date	No. Isolates
25 July	1	18 July	2
26 July	3	22 July	2
28 July	5	27 July	2
31 July	1	3 Aug.	3
2 Aug.	1	8 Aug.	4
4 Aug.	5	10 Aug.	1
7 Aug.	3	11 Aug.	1
9 Aug.	3	17 Aug.	1
11 Aug.	1		
14 Aug.	1		
16 Aug.	1		
18 Aug.	1		
30 Aug.	1		
	—		—
Total	27	Total	16

have been multiplied by the weekly percentage infection. The curve thus obtained may be considered as a rough index of mosquito infectivity. This curve is illustrated in Figure 14 in comparison with the C. tritaeniorhynchus population curve obtained from horse-baited traps and with the curve of the 1950 epizootic. If a 4-5 day incubation period in the natural infection in horses is assumed (as previously discussed) then the index of potential mosquito infectivity can be obtained for comparison with the 1950 epidemic curve and the human biting curve of C. tritaeniorhynchus. These curves are illustrated in Figure 15. Assuming a 10-24 day incubation period of Japanese B encephalitis infections in man (6) correlation is obtained once more, between the index of potential mosquito infectivity and the course of the epidemic. Caution must be used, however, in arriving at conclusions from this analysis. Examination of Table XXIV will show that the number of mosquito lots tested in the various weekly collection intervals shows wide variation. The weekly collection distribution of lots tested follows in general the rise and fall in the C. tritaeniorhynchus population during the summer. This results in a very small sample size for the early and late periods of the mosquito season. The limited number of isolations obtained during the first half of July and the latter half of August are, therefore, of questionable statistical significance. It should also be borne in mind that in Figure 14 a comparison is being made of mosquitoes collected only in horse-baited traps with virus isolations obtained from mosquitoes collected by several methods including horse-baited traps, cow-baited traps, pig-baited traps, and a variety of mosquito resting habitats. Further, while the mosquito collections were restricted to the city of Tokyo, epizootic data are based on cases occurring in the Kanto area, an area of considerably greater expanse. Similarly, in Figure 15 a comparison is being drawn between a mosquito population measured by human biting collection and virus isolations from mosquitoes collected by a wide variety of methods. These, and undoubtedly other factors, introduce a number of variables into the entire analysis.

The large series of isolates obtained in this work immediately raises the questions of possible laboratory contamination and of spontaneous infection with Japanese B encephalitis in the mice used in this work. It is believed that there is considerable evidence in the results obtained to date to substantiate the validity of the isolations. Some of the reasons which support the validity of this work are listed below:

1. Mosquito material was identified and tubed in a room in which no virus work had been previously performed.

Fig. 14 Relation of Mosquito Population and a Mosquito Infectivity Index to an Epizootic of JBE in Horses.

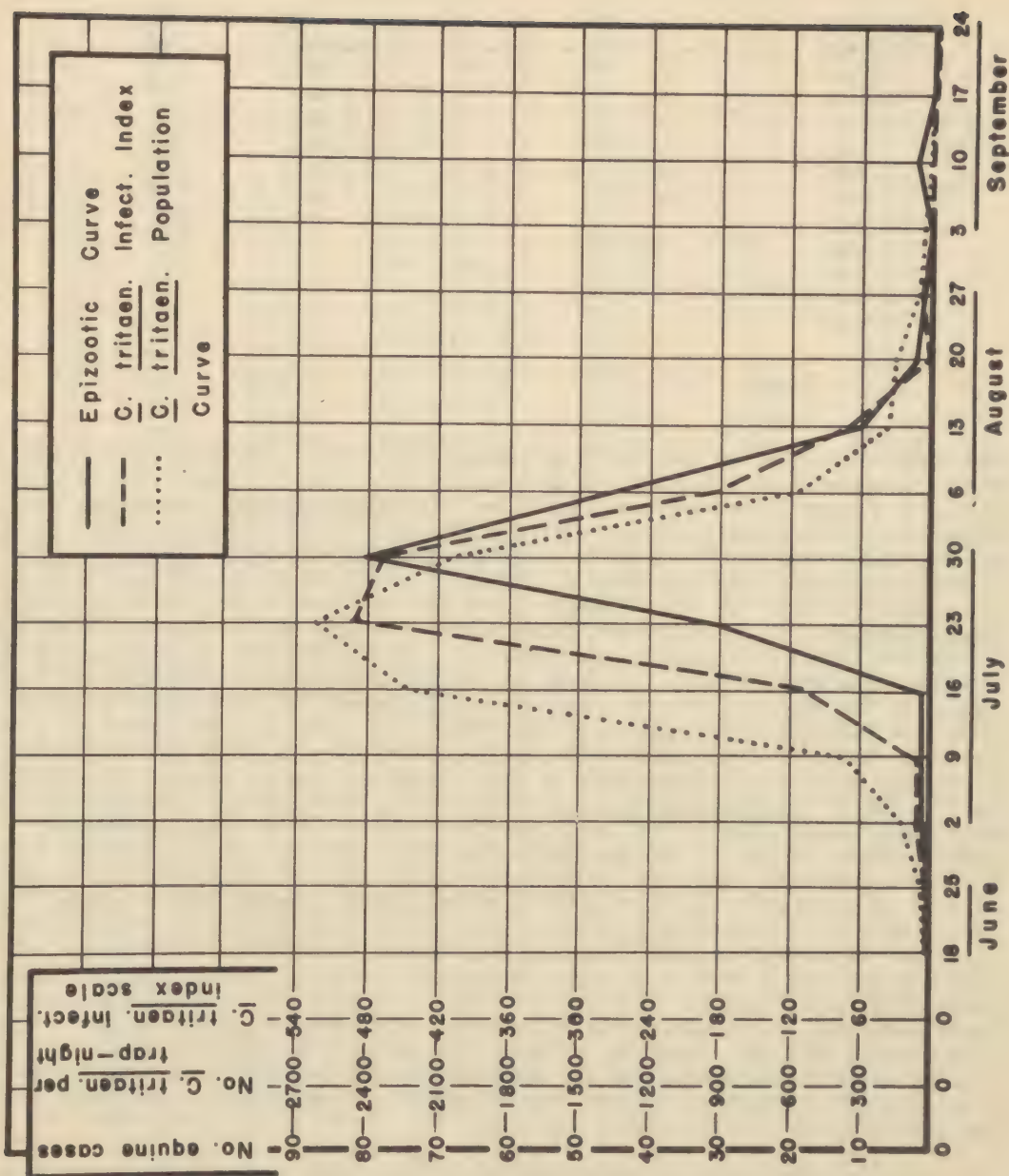


Fig. 15 Relation of Mosquito Population and a Mosquito Infectivity Index to an Epidemic of JBE.

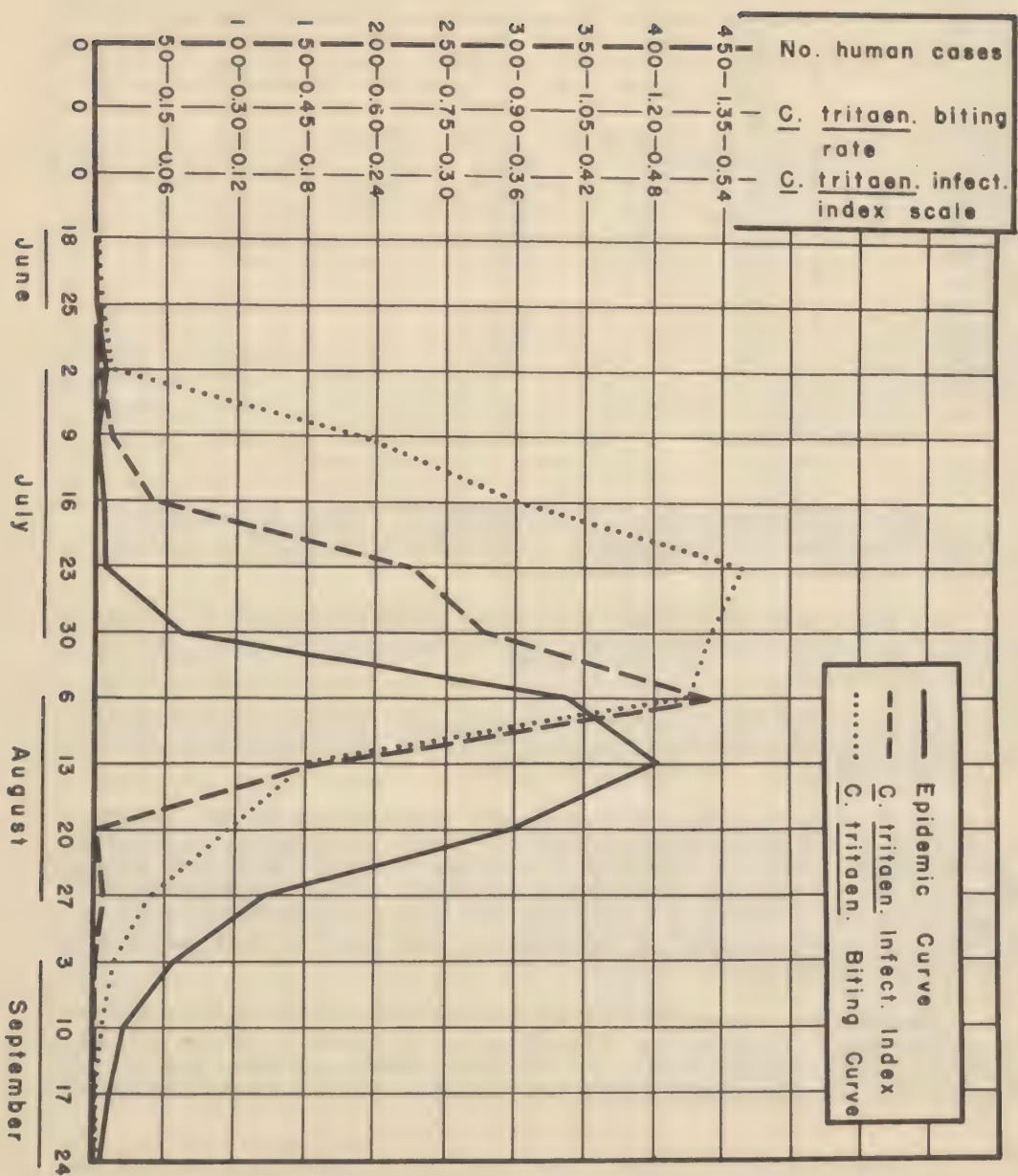


Table XXIV. Distribution of Virus Isolates from Mosquitoes by Date of Collection

<u>Week of</u>	<u>No. Lots Run</u>	<u>No. Isol.</u>	<u>% Isol.</u>
4 June	1	0	0
11 June	2	0	0
18 June	4	0	0
25 June	7	0	0
2 July	38	1	3
9 July	87	3	3
16 July	141	7	5
23 July	104	20	19
30 July	64	16	25
6 Aug.	46	19	41
13 Aug.	20	8	40
20 Aug.	15	0	0
27 Aug.	16	1	6
3 Sept.	6	0	0
10 Sept.	2	0	0
17 Sept.	1	0	0
Totals	554	75	13.5

2. Mosquitoes were identified by professionally trained entomologists before tubing and rechecked for verification of identifications prior to preparation of mosquito emulsions.

3. All virus isolates obtained from frozen mosquito material were obtained during late fall and winter, during a period when the only mosquito adults left in nature were in hibernation.

4. All virus isolates obtained by the Department of Entomology were prepared in a room in which no virus work had been previously performed, and in which no other virus work was being concurrently performed.

5. The 75 virus isolates obtained to date represent one or more isolates from 48 collections of Culex tritaeniorhynchus. Of the 48 collections, in 12 instances both the Virus and Entomology Departments have each obtained one or more isolates. This confirmation has been accomplished by the two groups working independently in different rooms, on widely divergent dates, and using mice obtained on different dates. No specific effort was made, in the course of this work, to give paired specimens to the two groups involved.

6. The isolates obtained to date are all confined to one mosquito species, Culex tritaeniorhynchus. Three hundred fifty-five lots of fresh and frozen mosquitoes totalling 18,952 female mosquito adults, and distributed among six other species and collected during the same period and in the same locations, gave consistently negative results.

7. The virus isolates obtained show a distinct aggregation by date of mosquito collection rather than by date of testing in the laboratory. It was found that a number of mosquito collections were "hot" collections regardless of the dates on which they were tested, while others were "cold" collections regardless of the dates on which they were tested.

8. All isolations except one were obtained from mice showing symptoms in the original group of mice inoculated with mosquito emulsion. One isolate was obtained after one blind passage, but this isolate was obtained from a "hot" collection from which another tube of mosquitoes had previously given an isolation. By going back to a preserved portion of the original mosquito emulsion, the virus was reisolated with symptoms appearing in the original group of mice inoculated.

9. During the entire course of this work only mice completely free of any symptoms of disease were used.

STOMOXYS SURVEY AND VIRUS ISOLATION ATTEMPTS: During 1950 a survey of the biting stable fly, Stomoxys calcitrans, was undertaken in conjunction with the studies on mosquitoes. The wide variety of hosts, including horses, cattle, pigs and man, from which this fly will take blood, mark it as a logical vector suspect. The only previously reported work on this species as a possible vector of Japanese B encephalitis was an attempt at virus isolations from approximately 1,000 specimens collected on Okinawa in 1945 by a U. S. Navy group (44).

In the course of the 1950 survey, 5015 Stomoxys calcitrans were collected, of which number 4,455 were tubed and frozen for subsequent virus isolation attempts. An additional 474 fresh Stomoxys were tested immediately after identification for the presence of virus. Other species occasionally taken were Musca hervei, a very common non-bloodsucking fly, and Tabanus mandarinus, a biting horse fly, seven specimens of which were frozen for subsequent virus isolation attempts.

Stomoxys did not appear in numbers until late June, the first collection being made on 27 June. A total of 70 collections were made, the last on 21 September. Early in these collections, an attempt was made to systematize collections so that population changes could be measured. It was found, however, that the species seemed to fluctuate widely in its resting places depending upon time of day, temperature, lighting and possibly other factors. Attempts at trapping the species using a modified Hodge trap (45) were uniformly unsuccessful. By midsummer, it became apparent that resting stations, biting collections, and trapping methods did not lend themselves readily to the measurement of population levels. However, from an examination of collection data, it is apparent that the largest collection of Stomoxys were obtained during the period of 13 August to 26 August.

In attempts at isolation of Japanese B encephalitis virus, 209 lots of frozen Stomoxys were run along with 3 lots of Tabanus mandarinus. In addition, 21 lots of fresh Stomoxys and 2 lots of fresh Tabanus mandarinus were run during the summer in immediate attempts at virus isolation. Fly material was tested in the same manner as mosquito material except that the size of the lots was smaller (25 or less per lot). The results were entirely negative, no isolations having been obtained. It is of interest to note that almost every fly emulsion produced heavy bacterial growth in tryptose-phosphate broth.

Summary - Studies were undertaken by the Department of Entomology during 1950 with the objectives of measuring mosquito populations for possible association with epidemics and epizootics of Japanese B encephalitis, of determining the degree of mosquito infection with Japanese B encephalitis virus and of determining host tropisms and biologies of mosquitoes. Other studies have included an overwintering survey of mosquitoes and virus isolation attempts on such material, and a survey to determine possible relationships of the biting stable fly, Stomoxys calcitrans, to epizootics and epidemics of Japanese B encephalitis.

DEPARTMENT OF VIRUS AND RICKETTSIAL DISEASES

ROUTINE: Routine tests performed are shown in Table I. As indicated therein, the major activities of this department center around Japanese B Encephalitis, influenza, and typhus. During 1950 there was a departure from past years in that Japanese health authorities having developed adequate facilities for serologic confirmation of encephalitis, only in rare instances were specimens from such cases submitted to this laboratory. For the first time, the major portion of encephalitis studies were concerned with clinical cases of the disease in American personnel.

Table I. Routine Tests Performed

Virus Complement-Fixation Test	6,543
Virus Neutralization Test	6,770
Virus Isolation Attempts	265
Typhus Complement-Fixation Test	495
Rickettsial Agglutination Test	98
Influenza Agglutination-Inhibition Test ..	1,326

JBE COMPLEMENT FIXATION: A total of 2,343 serum specimens were received on 721 patients for complement fixation test for JBE. In accordance with previously prescribed policy these included specimens on the majority of suspect central nervous system infections, since it has been previously established that exclusion of JBE infection is of definite value in all such cases, particularly during the epidemic period. It should be noted that despite year-round performance of such tests, no positive reactions are to be recorded for Japan except during the summer season. The complement fixation test follows the method described by Hammon (1) utilizing antigens prepared by the method of Espana and Hammon (2) and including parallel testing with antigens for SLE and WEE or EEE. Utilizing data previously collected (3) it was found necessary to establish arbitrary levels of complement-fixation titer to allow standardized interpretation of laboratory results by clinicians. These criteria attempted to consider the high degree of specificity exhibited by the test over the past years in this laboratory, the rare possibility of development of low titers in response to administered vaccine, the occasional difficulty in procurement of early negative sera, and the occasional inability to obtain later sera for demonstration of titer rises in patients returned to duty or evacuated to the Z.I. Accordingly, complement-fixation titers were classified as follows:

- (1) Negative - Failure of serum to fix complement beyond 50% in the initial tube having a final serum dilution of 1:4.
- (2) Suspicious - A titer of 3/ or 4/ in a dilution of 1:4.
- (3) Compatible - A positive reading (3/ or 4/) in a 1:8 dilution in the absence of an earlier lower or negative reaction. Such results are felt to shift the burden of proof to the clinician to disprove JBE.
- (4) Positive - A two tube rise (four-fold increase) in titer, or a sustained titer of 1:16 or higher. This is felt to establish the presence of JBE even in the absence of the clinically recognized disease.
- (5) Inadequate - Serum series not containing specimens both prior to the 21st and after the 34th day of disease are considered inadequate. Optimum periods appear to be at onset, 21st day, and 35th day of disease.

An attempt was made during the current season to obtain serum at ten day intervals from approximately 200 suspect cases in an attempt to arrive at a better evaluation of the rate and extent of complement-fixing titer rise and fall during the acute, convalescent, and rehabilitation periods. Analysis of these data is not yet complete but the criteria as outlined above still appear reasonable and adequate.

JBE NEUTRALIZATION TEST: A critical evaluation of the results of neutralization test is still to be completed. The difficulties in dependence on this test in vaccinated individuals, based on the experience of 1949, is presented in a later section of this report.

JBE VIRUS ISOLATION ATTEMPTS: Material was received from 56 autopsies on Americans for attempted isolation and identification of an etiologic viral agent. Of these cases on which brain tissue was received for virus studies 39 cases were histologically recognized as JBE. From these 39 cases virus isolates were obtained in 13 instances (33%). A summary of the isolates obtained, including the preliminary serologic identifications of the virus by complement-fixation test with antigens prepared from fourth or fifth mouse passage is shown in Table II. As has been recognized previously, there was some evidence of slight crossing with SLE hyperimmune serum but the extent of this reaction was minimal. All of the above antigens failed to react with either WEE or EEE sera.

Table II. Serological Results and Identification of Virus Isolates of Fatal Japanese B Encephalitis Suspects, Americans, 1950

Lab. No.	Case	Origin of In- fection	Source of Specimen	Date Rec'd	JBE CF Test on Virus Isolate	Days of Illness	Serology CF Reaction JBE	SLE	Neut. Index	JBE Immunization
V5-1824	LJ	Japan	361st SH	8/21	1:64(4f)	9	0	0	630	None
V5-1841	RW	Japan	USN Disp 3923	8/21	1:32(4f)	5	0	0	1000	ND
V5-2250	EF	Korea	118th SH	8/31	1:64(4f)	11	0	0	200	None
V5-2253	JT	Korea	8054th EH	9/1	1:64(4f)	5	-	-	-	None
V5-2286	HG	Korea	118th SH	9/2	1:128(4f)	3	0	0	320	None
V5-3298	AW	Korea	8054th EH	9/15	1:16(4f)	3	-	-	-	None
V5-3492	HB	Korea	Osaka AH	9/18	1:16(3f)	4	0	0	50	None
V5-3251	RL	Korea	8054th EH	9/18	1:32(4f)	3	-	-	-	None
V5-3558	LL	Korea	118th SH	9/19	1:16(3f)	5	0	0	320	ND
V5-3561	VY	Korea	118th SH	9/19	1:16(3f)	4	-	-	-	None
V5-3647	HG	Korea	118th SH	9/23	1:16(4f)	4	-	-	-	None
V5-3813	WB	Korea	118th SH	9/29	1:16(3f)	6	0	0	-	ND
V5-4473	MB	Korea	8054th EH	10/6	1:64(4f)	5	-	-	-	ND

- Test not conducted

ND JBE Immunization history not definite or conclusive

Virus isolation was also attempted on brain tissue from 10 fatal cases of suspect encephalitis in Japanese nationals. Two isolates were obtained and tentatively identified as JBE by complement-fixation. A single specimen of equine brain likewise yielded a virus which on fifth passage gave a positive complement fixation of 1:64 (4f) for JBE.

Japanese reports (4) concerning the reported recovery of JBE virus from various sites other than the central nervous system led to attempts at isolation from 15 spinal fluids, 8 blood specimens, and 4 fecal specimens; all results were negative on this material. This included many unexplained deaths and clinically typical bulbar poliomyelitis.

JBE IN OKINAWA: During 1950 a total of 14 suspect cases with no deaths in military personnel, and 31 cases with 13 deaths (case fatality rate 42%) were reported in the indigenous population (Fig. 1). Laboratory Results on limited serum samples submitted are presented in Table III and IIIA. This occurrence is consistent with those reported by Tigertt and Hammon (5) for the less severe years between 1945 and 1949.

Three additional cases of clinically diagnosed viral encephalitis appeared in military personnel, 2 in January and the third in April. No sera or inappropriately spaced samples were received. It will be noted (Table III) that 1 of 4 cases occurring in October has been serologically proven. Tigertt et al (5) likewise note more than 7 cases in natives reported in this "late" period, a prolongation of the period of case occurrence not usually found in Americans in Japan.

JAPANESE B ENCEPHALITIS IN OKINAWA — 1950 — BY WEEK OF ONSET

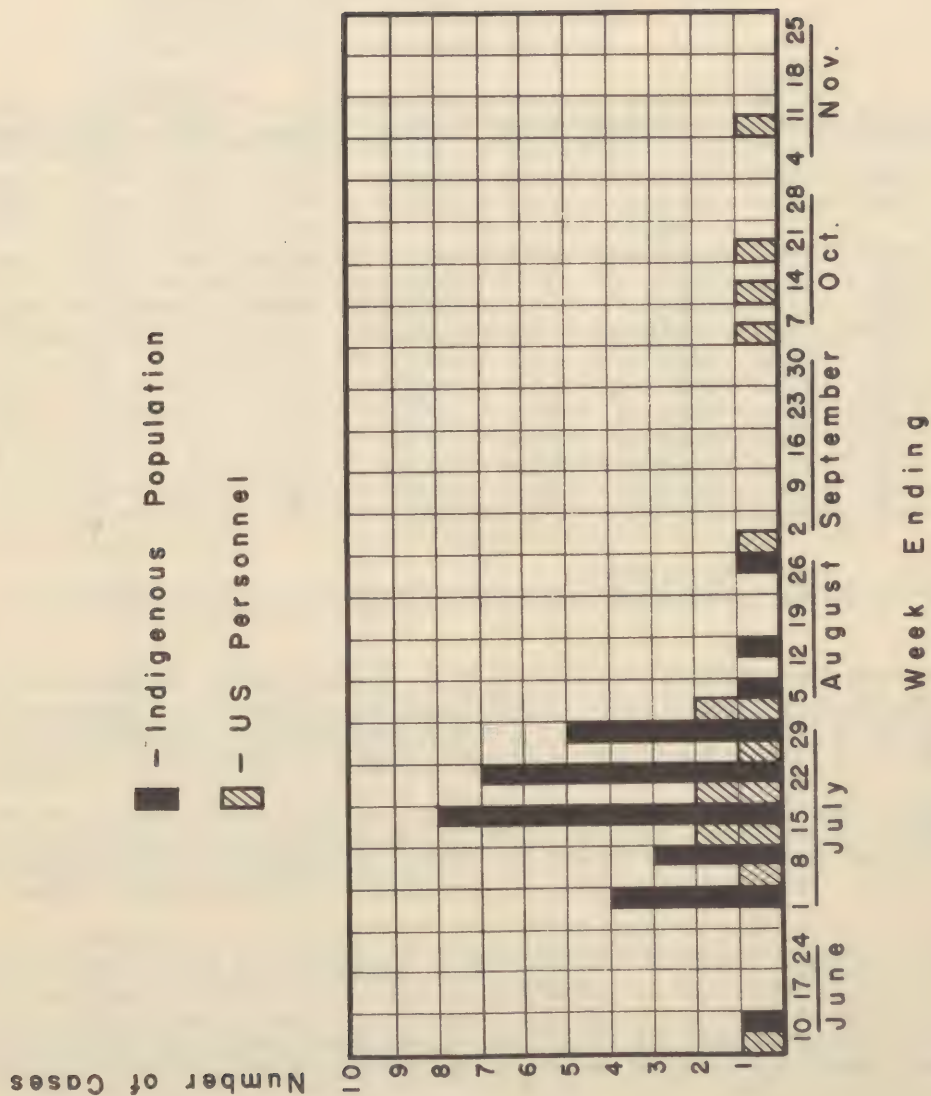


Figure 1

Table III. Clinical JBE in Americans, Okinawa 1950

<u>No.</u>	<u>Case</u>	<u>Onset</u>	<u>Immunization</u>	<u>Complement-Fixation Results</u>		
				<u>Positive</u>	<u>Suspicious</u>	<u>Inadequate</u>
1	JM	7 June	ND		0-1:4	
2	RWW	5 July	C		0-1:4	
3	DJ	11 July	ND			0
4	LV	15 July	ND			0
5	BP	21 July	ND	1:8-1:32		
6	GR	22 July	C			0
7	JT	23 July	ND			0
8	SF	30 July	C			0
9	RB	30 July	P		0-1:4	
10	SA	1 Sept.	C			0
11	WD	6 Oct.	O			0
12	CB	7 Oct.	ND			0
13	AR	17 Oct.	ND	0-1:8		
14	DH	8 Nov.	P			0

ND - JBE immunization history not definite or conclusive
 C - JBE immunization complete
 P - JBE immunization partial
 O - No JBE immunization

Table IIIA. Clinical JBE in Natives, Okinawa 1950

<u>No.</u>	<u>Age</u>	<u>Sex</u>	<u>Onset</u>	<u>Fatality</u>	<u>Complement-Fixation Results</u>				
					<u>Positive</u>	<u>Compatible</u>	<u>Suspicious</u>	<u>Negative</u>	<u>Inadequate</u>
1	10	M	9 June	+					0
2	18	M	26 June	+					0
3	13	F	28 June					0	
4	12	M	1 July	+					0
5	10	M	1 July	+					0
6	8	F	2 July				1:4		
7	3	M	4 July		0-1:8				
8	12	M	6 July				1:4		
9	20	M	9 July	+					0
10	33	F	10 July	+					0
11	5	F	11 July				1:4		
12	18	F	11 July	+					0
13	9	F	11 July		0-1:64				0
14	14	M	13 July				0-1:4		
15	8	F	13 July			1:8			
16	21	M	15 July						0
17	15	M	16 July		1:32				
18	21	F	16 July	+					
19	9	F	17 July					0	
20	9	M	19 July			1:4-1:8			
21	6	M	20 July				0-1:4		
22	11	M	20 July	+				0	
23	11	F	22 July			1:8			
24	17	M	23 July					0	
25	23	F	23 July	+					0
26	8	M	27 July	+					0
27	7	M	27 July				1:4		
28	19	M	29 July						0
29	7	M	29 July						0
30	12	M	7 Aug	+					0
31	48	M	26 Aug	+					0

JBE IN JAPAN: The first reported cases of JBE in American personnel had an onset on 7 August. The occurrence of cases in American personnel throughout Japan and in the indigenous population of Tokyo is represented in Figure 2. As indicated, the peak of the epidemic in military personnel was during the third week of August. Information from reports of the Public Health and Welfare Section, SCAP, reveals a total of 4903 cases in the Japanese with 1716 deaths. This represents a morbidity rate of 6.1 per 100,000 and a case fatality rate of 35%.

Some details of the individual cases occurring in American personnel in Japan is presented in Table IV.

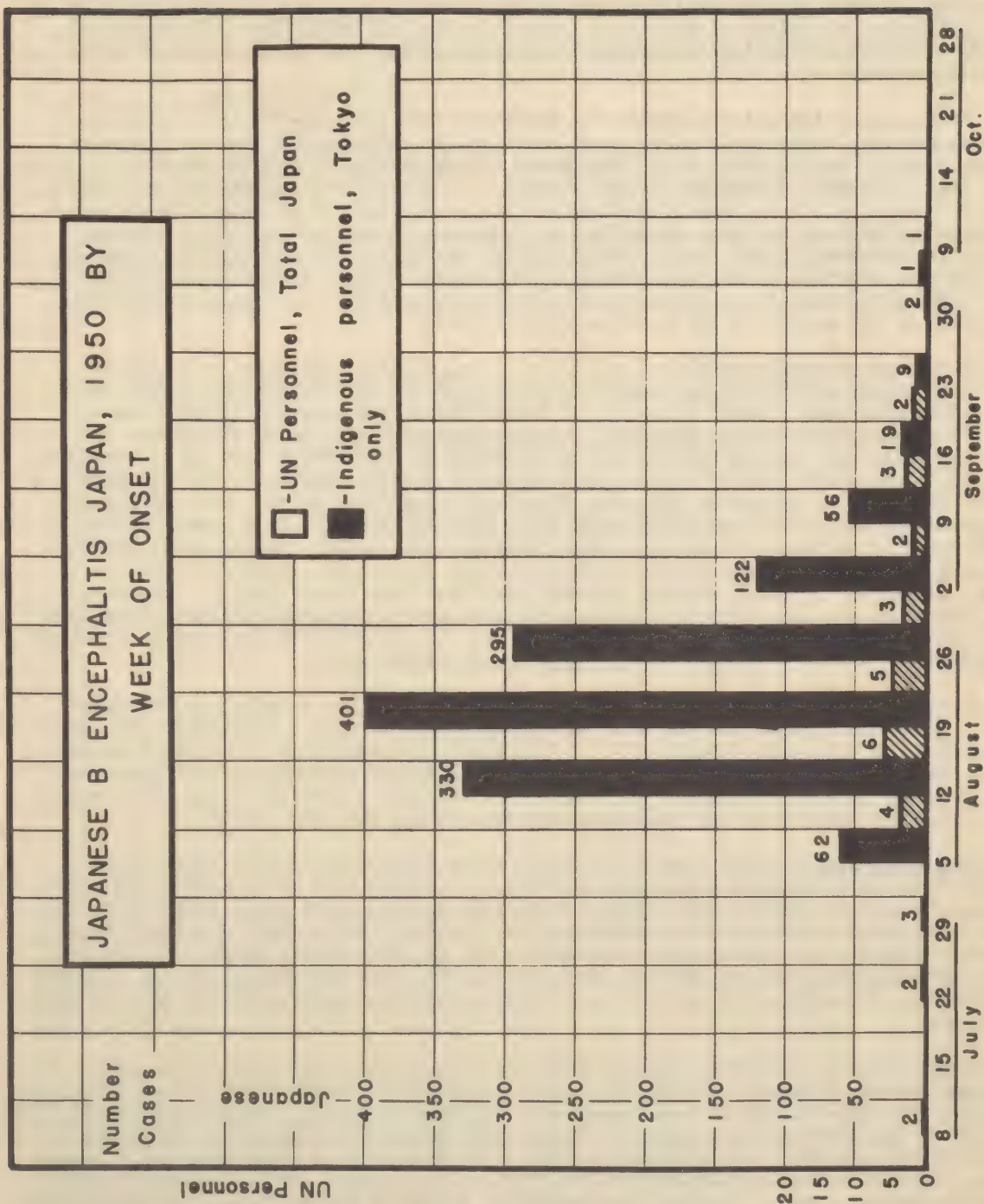
Table IV. Clinical JBE in UN Personnel, Japan 1950

No.	Case	Onset	JBE	Complement Fixation Results				Histologic Confirmation	Virus Isolation
			Immuniz.	Pos.	Susp.	Neg.	Inad.		
1	BB	8 Sept.	ND				0		
2	WB	17 Aug.	C			0			
3	LD	21 Sept.	C	1:16		0			
4	EE	17 Sept.	C			0			
5	AF	15 Aug.	C	0-1:32					
6	HG	26 Aug.	C			0			
7	BH	27 Aug.	P				0		
8	AH	15 Aug.	C	0-1:16					
9	WH	26 Aug.	O		0-1:4				
10	GH	16 Sept.	C		1:4				
11	CJ	22 Aug.	ND				0		
12	LJ	13 Aug.	P				0	+	+
13	RJ	7 Aug.	C	1:4-1:32					
14	IK	4 Sept.	C	0-1:32					
15	VK	8 Aug.	O				0	+	-
16	CM	31 Aug.	ND			0			
17	RMc	2 Oct.	C			0			
18	RM	20 Aug.	O	0-1:8					
19	HP	27 Aug.	O	0-1:16					
20	JP	23 Aug.	P					+	-
21	AP	14 Sept.	P				0		
22	OR	11 Sept.	O	0-1:4					
23	LS	17 Aug.		1:16-1:128					
24	RW	16 Aug.	ND				0	+	+
25	WW	13 Aug.	P				0	+	-
26	PW	7 Aug.	C	1:4-1:128					

ND, C, P, and O as in Table III

JBE IN KOREA: A recent review (6) has suggested the long standing periodic occurrence of Japanese B Encephalitis in Korea. Whereas the disease has been recognized in Japan since 1924 with outbreaks reaching major epidemic proportions in 1924, 1935, and 1948, similar epidemic cycles have probably occurred in Korea in 1924, 1934, 1935, 1939, and 1949. In 1946 four cases occurred in American soldiers, one fatal case being proven by virus isolation, the others serologically (7). Serologic surveys that same year of indigenous persons and animals indicated wide dissemination of JBE virus (8). During August and September 1949 a severe epidemic resulted in 5,548 cases (1,283 in Seoul) with 2,429 reported deaths, and was identified as being due to JBE virus (6).

The introduction of American and other susceptible U.N. troops under combat conditions provided a fertile field for appearance of this disease in such forces. Security reasons prevent publication in this report of many details, and within such restrictions, plus those related to difficulties encountered in epidemiologic investigations under conditions encountered in rapid transition to a state of war, an attempt will be made to present data concerning JBE in Korea in 1950. A more complete and final report will follow.



Week Ending
Figure 2.

The first case had a date of onset on 20 July and during the next two and a half months approximately 350 cases were clinically suspected. Fortunately, a prepared plan for epidemiologic and laboratory investigation already existed for cases expected in Japan and required only amplification to allow attempted coverage of this new area. It should be realized that difficulties in the tactical situation interposed many impediments to attempts at a thorough collection of data. The difficulties of initial logistic support plus tactical reverses undoubtedly are an explanation for some deficiency in individual methods of protection against mosquitoes. It should be born in mind that during this particular period protection from missiles was of paramount importance.

The disproportion between numbers of casualties and available medical facilities in Korea was one factor in formulation of a policy for rapid evacuation of clinically suspect cases of encephalitis from Korea prior to the fastigium of the disease in order that the detailed symptomatic and nursing care required during periods of coma in severe cases were more readily available. The great majority of such patients who were transportable at the time of arrival at Pusan were moved to the 118th Station Hospital in Southern Japan. Subsequently, during convalescence all remaining cases of this disease save those also requiring major surgical care were concentrated at the 361st Station Hospital for neuropsychiatric evaluation and for preliminary rehabilitation prior to the return of the great majority to duty.

In conformity with established policy in Japan, all cases admitted to hospitals with or subsequently developing suspect infections of the central nervous system were reported by radiogram. Cases dying at the 8054th Evacuation Hospital in Korea were reported by phone and autopsy protocol. During the period 20 July to 20 October 347 cases with clinical findings suggesting an infection of the central nervous system were included in the study group. A subsequent review of clinical abstracts was ultimately used as the means of selecting 299 cases from Korea considered on the basis of our experience to be clinically consistent with JBE. This entailed the well known difficulty of differentiating poliomyelitis from this disease. In general, typical cases showing the usual prodromal fever, mental confusion, coma, a picture of changing abnormal reflexes, and marked recovery without residual paralysis gave no difficulty. However, past experience has indicated that occasional cases showing typical findings of poliomyelitis have been due to the virus of JBE and it is felt that in the excluded cases there undoubtedly were some instances of infection by the latter agent.

The clinical picture has been presented by others in great detail. As indicated before, while the typical picture of severe encephalitis affords a relatively easy clinical problem in diagnosis, roughly 70% of this group of cases can be considered as moderate or mild. In such instances the diagnosis becomes increasingly more difficult and quite possibly this condition was responsible for at least a portion of the large number of soldiers diagnosed fever of undetermined origin during the same period.

Incubation Period - The length of time in Korea preceding onset is known in 207 cases. Onset of symptoms are recorded as early as 7 and 10 days after arrival in Korea. However, these individuals spent six and seven days in Japan just prior to going to Korea. One case entered Korea directly from the United States without docking in Japan. The first case was not serologically confirmed, but the second case showed a diagnostic titer. He had never been vaccinated. Hence it appears that incubation periods as short as seven days probably occur and that incubation periods as short as twelve days undoubtedly occur.

Twenty-seven of the 299 cases had their onset in Japan following evacuation from Korea for other causes. Inasmuch as JBE has never previously been contracted in fixed hospitals in Japan, it is probable that these cases were infected in Korea. Onsets ranged from day of transfer to 14 days after arrival in Japan. One such case developed encephalitis 21 days after the onset of infectious hepatitis, or 14 days after arrival in Japan. A compatible titer rise was demonstrated by the 12th day of disease. He had received an incomplete JBE vaccine series. Another case sustained a gunshot wound 17 days before the onset of encephalitis and was evacuated 6 days later, strongly suggesting a minimum incubation period of 11 days. A diagnostic CF titer was demonstrated. The patient had never received JBE vaccine.

It is impossible to establish on the basis of these data an incubation period range, however a 7 to 14 day span is suggested.

Laboratory Findings in Non-Fatal Cases - Of 211 spinal fluid cell counts on 194 patients between the first and fifth day of disease, total cell counts ranged from 0 to 3,350 WBC/cu.mm. The mean total count was 277/cu.mm. The mean percentage of lymphocytes on this same group was 65%. Of 160 recorded spinal fluid examinations performed between the sixth and tenth day of disease total counts ranged from 0 to 1,203 WBC/cu.mm. with a mean of 131, and the mean percentage of lymphocytes was 85%. In several instances, repeated examinations showed disappearance of an initial polymorphonuclear reaction as high as 40%.

Findings in the peripheral blood were not particularly noteworthy with the exception of the fact that of 260 patients, 45% showed a WBC count of over 12,000/cu.mm. and 28% exceeded 15,000.

Of the 299 cases adequate serum specimens were received in 237 instances. Using a standardized complement-fixation test for JBE with final serum dilutions of 1:4, 1:8, 1:16, etc. results are as shown in Table V. It will be noted that in 16% of the clinically selected cases, no reaction of 50% or more occurred in the dilution of 1:4 or higher.

Table V. Results of Complement Fixation Tests on 237 Clinical Cases of JBE from Korea

Cases Showing Titer Rise by Tube

1 tube rise	63
2 tube rise	60
3 tube rise	43
4 tube rise	16
5 tube rise	2

Maintained CF Titer in Cases Showing No Titer Rise

0	38
1:4	6
1:8	4
1:16	2
1:32	2
1:64	1

Total 237

Fatalities - Deaths occurring in the group of 299 clinically accepted cases amounted to 30 (Table VI). Of 40 deaths considered in the originally reported group from Korea 28 showed the typical histologic picture of JBE and are discussed in a separate part of this report, (see Pathology Department). In the other two cases, the clinical picture was typical, but one (GS) is histologically poliomyelitis and the other (RA) is compatible but not typical of JBE. Of the 10 deaths excluded histologic examination revealed 4 poliomyelitis, 2 fat embolism, 1 hepatitis, 1 meningitis, 1 focal hemorrhage of cerebellum, 1 undetermined but no encephalitis.

INAPPARENT INFECTIONS: The established fact of a high degree of immunity in Japanese adults has been previously noted. Such immunity has been considered the result of naturally acquired inapparent infections. The opportunity to demonstrate such inapparent infections in troops in Korea was undertaken on a limited scale. During the period 25 September to 10 October blood was collected from patients in hospitals in Tokyo. These individuals were selected on the basis of the following:

(1) Possession of an immunization record.

(2) Exposure in Korea for at least 1 to 2 weeks during the period 1 August to 30 September.

Table VI. 30 Deaths in U.N. Personnel from Clinical JBE Contracted in Korea

Korea Case No.	Name	Date of Onset	Death by Day of Disease	Unit	JBE Immun- ization	Hist. Confirm.	Virus Isol.	CF Titer and Day of Dis.
1	RA	26 Aug	17	5 RCT	ND	?	-	0 17
13	HB	12 Sept	6	2 Div	0	/	/	0 4
15	GB	5 Sept	6	2 Div	0	/	-	0 4
17	JB	11 Sept	10	24 Div	ND	/	-	1:8 5
19	WB	20 Sept	7	1 Cav	ND	/	/	0 5
32	EB	28 Sept	6	2 Div	ND	/	/	NT
49	GC	20 Sept	6	1 Mar	ND	/	-	0 6
56	JC	2 Sept	7	24 Div	0	/	-	NT
73	FD	12 Sept	6	2 Div	0	/	-	NT
74	HD	4 Sept	7	1 Cav	C	/	-	NT
83	AF	6 Sept	94	1 Mar	ND	/	-	1:8 34
86	HF	9 Sept	7	2 Div	0	/	-	0 6
87	EF	19 Aug	12	AF	0	/	/	0 11
92	JF	26 Sept	4	1 Mar	0	/	-	NT
99	HG	16 Sept	4	2 Div	0	/	/	NT
112	HG	29 Aug	2	2 Div	0	/	/	NT
143	EJ	16 Sept	4	1 Cav	ND	/	-	NT
151	RK	23 Sept	6	2 Div	ND	/	-	0 6
153	LL	12 Sept	6	Misc.	ND	/	/	0 4
158	RL	13 Sept	4	2 Div	0	/	/	NT
190	MO	27 Aug	7	24 Div	ND	/	-	0 17
196	HP	16 Sept	7	24 Div	ND	/	-	0 5
199	DP	15 Sept	8	2 Div	0	/	-	0 7
201	LP	14 Sept	3	1 Mar	ND	/	NT	NT
236	GS	22 Aug	7	2 Div	0	-	-	0 6
255	JT	25 Aug	7	1 Mar	0	/	/	NT
268	JV	19 Sept	6	1 Cav	ND	/	-	NT
276	RW	10 Sept	6	2 Div	0	/	NT	NT
285	AW	11 Sept	3	24 Div	0	/	/	NT
292	VY	16 Sept	2	2 Div	0	/	/	NT

3. Evacuation from Korea for any reason other than suspect central nervous system infection.

Approximately 400 such patients were selected with an attempt being made to balance roughly the total number between those immunized and those non-immunized with JBE vaccine. In addition to JBE immunization data, information was collected as to unit to which assigned in Korea, date of arrival in Japan, date of arrival in Korea, and date of return to Japan from Korea. The sera collected from this group of cases were stored in dry ice chests and were tested by complement fixation and neutralization test during the last two months of the year.

As mentioned previously, it was generally assumed that the personnel of the 24th Division, 25th Division, and 1st Cavalry Division had been rather completely immunized in the past and in the current year during their assignment in Japan. The 2nd Division arriving in Korea direct from the U.S. was assumed to be composed of non-immunized individuals. Using the criteria applicable in current JBE immunization programs, these cases were evaluated as follows:

(1) Completely Immunized - A series of 3 injections in the current season or a minimum initial series of 2 injections in any single previous season followed by an annual stimulating dose including 1950.

(2) Partially Immunized - Individuals receiving less than the above stated amounts.

(3) Non-Immunized - Individuals whose records showed no history of administration of JBE vaccine in this or in previous years.

Admitting that this survey group may represent appreciable dilution of original personnel comprising each of the four major tactical units, and realizing the small size of the sample represented, it is found that between 26 and 36 percent of individuals in this group from the "immunized" Divisions and 84% from the "non-immunized" Division had actually received no JBE vaccine (Table VII).

Table VII. Degree of Immunization of Units in Inapparent Infection Survey

	<u>Complete</u>	<u>Partial</u>	<u>None</u>	<u>Total</u>
24th Div.	20 (43%)	15 (32%)	12 (26%)	47
25th Div.	31 (41%)	18 (24%)	27 (36%)	76
1st Cav Div.	32 (36%)	28 (32%)	28 (32%)	88
2nd Div.	3 (3%)	13 (13%)	83 (84%)	99
Misc.	16 (22%)	18 (25%)	38 (53%)	72
				<u>382</u>

Tabulation of the results of the JBE complement-fixation shows that 11 to 15 per cent of this exposed group had titers of 1:4 or higher (Table VIII). In our past experience administration of JBE vaccine will not result in the production of CF titers unless the individual has had previous experience with the virus. It will be noted that 12 percent of the individuals who received no vaccine still showed a positive CF reaction. Of this non-immunized group with positive CF only 2 individuals had had exposure in Japan in previous seasons. Arbitrarily assuming the months of July, August, and September as periods of possible previous exposure, this group of cases is tabulated in greater detail in Table IX.

Table VIII. Inapparent Infection as Indicated by Complement Fixation Test

<u>JBE</u> <u>Immunization</u>	<u>Total</u> <u>Tested</u>	<u>1:32</u>	<u>1:16</u>	<u>1:8</u>	<u>1:4</u>	<u>Negative*</u>
Complete	102	0	2	7	15 (15%)	87
Partial	92	0		5	10 (11%)	82
None	191	1	3	10	23 (12%)	168
	<u>385</u>					

* Includes cases showing 1/ and 2/ reactions in 1:4 dilution as follows: complete 1, Partial 1, none 6.

As noted in Figure 3, the divisional units in diminishing order of clinical cases of JBE were: 2nd Division, 25th Division, 24th Division, and 1st Cavalry Division. Table IX reflects inapparent exposures as determined by CF test with regard to unit, degree of immunization, and period of exposure. Again realizing the small sample constituting this survey, it appears that inapparent infections by unit in diminishing order of frequency are 25th Division, 2nd Division, 1st Cavalry Division, and 24th Division. Attempting to explain the frequency by unit shown in Table I, the average number of days exposure in Korea for each man is shown by category and unit. The greater period of average exposure of the 25th Division appears a possible explanation of the lower frequency of inapparent infections in the "non-immunized" 2nd Division. Comparison of the relative frequency of inapparent infections in the 25th Division with each of the other two "immunized" Divisions suggests that geographic location may have been an extremely important factor. As will be recalled from newspaper accounts of the period of the Pusan perimeter the general location of these divisions from South to North was 2nd Division, 25th Division, 24th Division, and 1st Cavalry Division.

Table IX. Non-Immunized Soldiers Showing a Positive Complement Fixation Test

<u>Case</u>	<u>Prior Svc*</u> <u>in Japan</u> <u>(Months)</u>	<u>Current Svc*</u> <u>in Japan</u> <u>(weeks)</u>	<u>Current Svc</u> <u>in Korea</u> <u>(Weeks)</u>	<u>Complement</u> <u>Fixation</u> <u>Titer</u>
RH		5	4	1:4
LH		4	5	1:4
GS		1	4	1:4
RO		4	5	1:4
GM		3	6	1:4
PB		1	7	1:4
BE		1	9	1:4
LD		2	3	1:8
AM		2	4	1:8
JJ		1	5	1:8
JL		1	7	1:8
JA	6	3	9	1:8
RS		0	9	1:8
EP		3	5	1:16
WC		3	5	1:16
SD		2	9	1:32
JJ		0	10	1:8
CA		1	7	1:4
JM		4	5	1:4
DE		3	6	1:4
DG	3	4	9	1:4
LH		0	3	1:4
CM		2	3	1:4

* Service counted only during epidemic periods, arbitrarily set as July, August, September

Table X. Influence of Unit, Immunization, and Exposure in Inapparent Infection as Determined by Complement Fixation

<u>Unit</u>	<u>CF</u>	<u>JBE Immunization</u>			<u>Total</u>		<u>Ave. Days</u>
		<u>Complete</u>	<u>Partial</u>	<u>None</u>	<u>No.</u>	<u>%</u>	<u>Exposure</u>
24th Div.	Pos.	1	0	0	1	2.1	49
	Neg.	19	15	12	46	97.9	45
25th Div.	Pos.	11	3	4	18	23.7	53
	Neg.	20	15	23	58	76.3	41
1st Cav	Pos.	3	2	0	5	5.7	57
	Neg.	29	26	28	83	94.3	46
2nd Div.	Pos.	0	0	16	16	16.2	43
	Neg.	3	13	67	83	83.8	38
Misc.	Pos.	0	1	3	4	5.6	49
	Neg.	16	17	35	68	94.4	37

In using the neutralization test in previous surveys in this area on immunized and non-immunized Americans, and in large scale surveys of Japanese, experience dictated acceptance of the following in interpretation of survey results:

JAPANESE B ENCEPHALITIS IN JAPAN AND KOREA - 1950 - IN UN PERSONNEL (EXCL. ROK) BY DATE OF ONSET

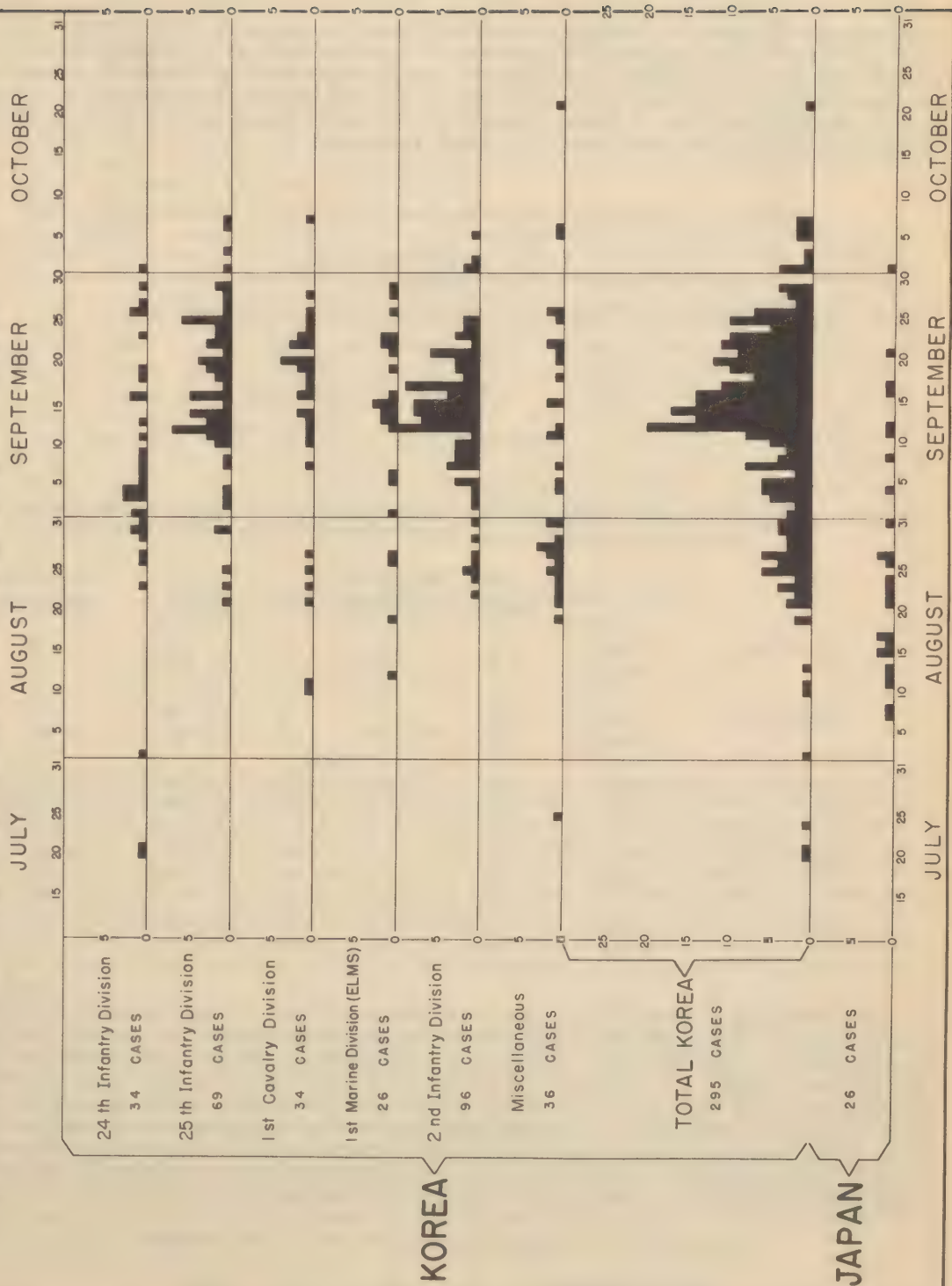


FIGURE 3

<u>Logs Protection</u>	<u>Neutralization Index</u>	<u>Interpretation</u>
<1.0	10	Negative
1.0-1.6	10-49	Equivocal
>1.6	50 and greater	Positive

Considering the equivocal category to be just that, an analysis of 315 of the survey sera are found to show appreciable amounts of virus neutralizing antibodies in approximately 50% of cases studies regardless of previous JBE vaccine (Table XI). Detailed breakdown by unit is presented in Table XII. Also as suggested by results of CF testing, it would appear that a factor, possibly geographic location, may be as important as vaccination in protection from inapparent infection.

Table XI. Inapparent Infections as Indicated by Neutralization Test

<u>JBE Immunization</u>	<u>Total Tested</u>	<u>Logs Protection (Cumulative)</u>					<u>1.7</u>
		<u>4</u>	<u>3</u>	<u>2</u>	<u>1.7</u>		
Complete	90	14	31	37	44(49%)		46
Partial	76	8	28	31	32(42%)		44
None	149	16	77	83	85 (57%)		64
Total Tested							315

Table XII. Influence of Unit, Immunization, and Period of Exposure on Inapparent Infections as Determined by Neutralization Tests

		<u>JBE Immunization</u>			<u>Total</u>		<u>Ave. Days Exposure</u>
		<u>Complete</u>	<u>Partial</u>	<u>None</u>	<u>No.</u>	<u>%</u>	
24th Div.	Pos.	7	3	3	13	37.1	54
	Neg.	7	9	6	22	62.9	43
25th Div.	Pos.	19	7	8	34	56.7	47
	Neg.	9	7	10	26	43.3	44
1st Cav.	Pos.	12	8	3	23	29.1	47
	Neg.	20	17	19	56	70.9	48
2nd Div.	Pos.	1	8	58	67	79.8	42
	Neg.	1	3	13	17	20.2	35
Misc.	Pos.	5	6	13	24	42.1	47
	Neg.	9	8	16	33	57.9	32

Fortunately, 53 Republic of Korea soldiers were found in one hospital visited. Practically all these had been evacuated during a period similar to that of the Americans included in the survey. Of the 53 sera examined by CF test no positive reactions were found. Furthermore, all survey sera were tested without knowledge of any information as to source, and these ROK bloods were invariably run in groups of 8 to 10 as part of a total days run of approximately 60 total sera tested. In marked contrast to this are the results of neutralization tests on 48 of the original 53 sera. Of this small group 90% show 1.7 logs protection or more, and actually 83% of the total tested showed 3 or greater logs protection (Table XIII).

Table XIII. Neutralization Tests on 48 ROK Soldiers

<u>Logs Protection</u>	<u>3</u>	<u>2</u>	<u>1.7</u>	<u>< 1.7</u>
<u>No. of Cases</u>	40	2	1	5

Vaccine Evaluation - In general, the 24th and 25th Infantry Divisions and the 1st Cavalry Division were immunized to some degree, having been on occupation duty in Japan prior to shipment to Korea. Members of the First Marine Brigade arriving in Korea direct from the U.S. received an initial inoculation of vaccine only. The 2nd Infantry Division and 5th RCT, plus other smaller units arriving in Korea direct from areas other than this theater received no JBE vaccine. Considering the rates shown in the 2nd Division would definitely suggest the vaccine as of value in preventing appearance of clinical cases of the disease (Fig. 3). The opportunity for a definitive evaluation of JBE vaccine was realized early in the course of the current epidemic by local observers and by the Virus and Rickettsial Commission of the Armed Forces Epidemiological Board, Colonel A. P. Long, Preventive Medicine Consultant, Far East Command, Dr. Grant Taylor, Associate Member of the Virus Commission, and Dr. Ross Gauld of the AMSGS assisted in directing the local epidemiologic investigation and in obtaining results to allow evaluation of vaccine efficacy. Final statistical tabulation of results and information as to effectiveness of the vaccine will be reported later.

JBE IN KOREANS: Accurate data on the civilian population of war-torn Korea has been non-existent. Encephalitis even in relatively large amounts would have attracted little attention in a population undergoing mass displacement with almost all medical and government facilities required for their war effort, and with malnutrition and other diseases being of more immediate and recognizable importance. However, in a small town on the southern coast between Masan and Pusan several suspect cases were recognized, and under the direction of Colonel James Gordon, Preventive Medicine Officer, Eighth Army, paired serum specimens were obtained on 7 such cases. In three individuals with no reaction in the first specimen, CF titers of 1:4, 1:8, and 1:16 appeared later. The other 4 cases showed no reaction and subsequent specimens were not obtained. One clinical case appeared in a 19 year old ROK soldier recently arrived from Korea for duty with U.S. troops. Exact dates of arrival and of onset of symptoms are unknown. On 7 September he was admitted to the hospital in coma with a temperature of 105°F, stiff neck and pleocytosis of 173/cumm, all lymphocytes. A series of 4 blood specimens taken prior to his evacuation to Korea showed the following:

<u>Date of Collection</u>	<u>Complement Fixation</u>	<u>Neutralization Index</u>
7 Sept.	0	160
12 Sept.	0	790
18 Sept.	0	2500
30 Sept.	1:4 (4f)	4000

CONGLUTINATING COMPLEMENT ABSORPTION TEST IN JBE: An investigation was conducted on the phenomenon of conglutination as a possibility for the application to a diagnostic study of Japanese B encephalitis and other viral encephalitides. This test is based on the observation that when heat inactivated bovine serum and fresh (unheated) horse, cat or pig serum are added to a suspension of sheep red cells, the cells clump together or conglutinate. Conglutination may be considered analogous to hemolysis in the hemolytic complement-fixation test. Sensitivity is largely sacrificed for specificity in complement-fixation test, and modifications, such as alternations of the units of complement, varying the time and temperature of the incubation period, have not proved entirely satisfactory.

The technique of the conglutinating complement absorption test (CCAT) followed that of Hole and Coombs (9) with certain modifications as set forth by Wolfe and Kornfeldt (10). The hemolytic complement-fixation test run for comparative studies was the procedure described by Casals et al (11). There were many difficulties encountered during this study, i.e. the source of complement utilized was horse serum and it was difficult to find horses which did not have complement-fixing antibodies due to inapparent infection with the virus of Japanese B encephalitis. The conglutinin was obtained from bovine sera and it was recognized early that the conglutinin titre of individual bovines was quite variable. At least four conglutinating doses in 0.4 ml. of serum is required in the test proper.

Typical results of comparative studies using both HCF and CCAT procedures are shown in Tables XIV, XV, XVI, and XVII.

Table XIV. Comparison of Homologous Conglutinin Complement Absorption (CCAT) and Hemolytic Complement Fixation (HCF) Titer with Japanese B Encephalitis, St. Louis and Western Equine Encephalitis Antigens in 30 Hyperimmune Guinea Pig Sera

CCAT Titre	No. of Sera with HCF Titre										
	<u>Neg.</u>	<u>1:4</u>	<u>1:8</u>	<u>1:16</u>	<u>1:32</u>	<u>1:64</u>	<u>1:128</u>	<u>1:256</u>	<u>NMB</u>	<u>AC</u>	<u>Total</u>
Negative	2*										2
1:4			1	1	1						3
1:8											0
1:16						1					1
1:32						2					2
1:64					2	2	1				5
1:128								1			1
1:256						1	4	3			8
1:512								1			1
NMB											0
AC	<u>1</u>	<u>—</u>	<u>1</u>	<u>—</u>	<u>2</u>	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>	<u>3</u>	<u>7</u>
Total	3	0	2	1	5	6	5	5	0	3	30

* A series of 17 guinea pigs tested prior to immunization were all negative for encephalitis antibodies with CCAT and HCF tests

Table XV. Comparison of Conglutinin Complement Absorption (CCAT) and Hemolytic Complement Fixation (HCF) Titre with Japanese B Encephalitis Antigens in 62 Horse Sera

CCAT Titre	No. of Sera with HCF Titre										
	Neg.	1:4	1:8	1:16	1:32	1:64	1:128	1:256	NMB	A.C.	Total
Negative	10										10
1:4	2								1		3
1:8	1									1	2
1:16	6	3	2							5	16
1:32	2	2	7								11
1:64		1	2								3
1:128				5							5
1:256			1	4	2						7
1:512					3						3
1:1024						1	1				2
1:2048											0
NMB											0
A.C.											0
Total	21	6	12	9	5	1	1	0	1	6	62

Summary - The CCAT using horse serum as a complement source is more sensitive than is the present HCF test employed in the Japanese B encephalitis horse serum system.

Table XVI. Comparison of Homologous (JBE) and Heterologous Titres (SLE) as Determined by CCAT and HCT Tests in Horse Sera

No. Specimen	JBE	SLE	Titre with		
			WEE	NMB	SAL
V5-146					
CCAT	1024	32	-	-	-
HCF	64	64	-	-	-
V5-147					
CCAT	128	16	-	-	-
HCF	32	16	-	-	-
V5-90					
CCAT	246	32	-	-	-
HCF	32	32	-	-	-
H-7					
CCAT	32	-	-	-	-
HCF	4	-	-	-	-
V5-116					
CCAT	64	-	-	-	-
HCF	8	-	-	-	-
V5-85					
CCAT	32	-	-	-	-
HCF	4	-	-	-	-
V5-91					
CCAT	256	16	-	-	-
HCF	16	32	-	-	-
V5-93					
CCAT	32	-	-	-	-
HCF	8	16	-	-	-
V5-41					
CCAT	8	-	-	-	-
HCF	-	8	-	-	-
V5-143					
CCAT	512	32	-	-	-
HCF	32	-	-	-	-
V5-146					
CCAT	1024	32	-	-	-
HCF	64	64	-	-	-
V5-147					
CCAT	128	16	-	-	-
HCF	32	16	-	-	-
H-8					
CCAT	8	-	-	-	-
HCF	4	4	4	4	4
H-12					
CCAT	16	-	-	-	-
HCF	4	4	4	4	4

Table XVII. Comparison of Conglutinin Complement Absorption (CCAT and the Hemolytic Complement Fixation (HCF) Titres with JBE Antigen in 96 Human Sera

CCAT Titre	No. of Sera with HCF Titre							A.C. ***	Total
	Neg.*	1:4	1:8	1:16	1:32	1:64	NMB**		
Negative	26	8	10	2					46
1:4	6	5	2	2					15
1:8	1	2	1	1					5
1:16		3	2	1	1				7
1:32				2	1				3
1:64				1					1
1:128									0
NMB									0
A.C.	9	5	1	2				2	19
Total	42	23	16	11	2	0	0	2	96

* Less than 1:4

** Normal Mouse Brain

*** Anticomplementary

POLIOMYELITIS: Twenty-one specimens for virus study were received from American cases clinically diagnosed as poliomyelitis. These included 11 brain tissues, 5 spinal fluids, and 4 fecal specimens.

These specimens were handled in the same manner as those suspected of Japanese B encephalitis, as the main purpose of the determinations was to aid in differential diagnosis from infection with Japanese B encephalitis. No monkeys were inoculated. Isolation attempts were confirmed entirely to suckling and three week old white mice. All results were negative in this group.

COXSACKIE VIRUS: Because of the number of mild febrile illnesses reported among occupation personnel and the frequent diagnosis of non-paralytic poliomyelitis, it is not unreasonable to assume that a virus of the Coxsackie type may be responsible for these outbreaks. In addition, it has been reported by numerous Japanese workers (12) that the virus of Japanese B encephalitis has and can be isolated from the stools of individuals affected with that virus, although no American worker has confirmed these results. The significance attached to the isolation and identification of this agent during the period of acute illness in non-fatal cases is obvious.

Acting upon this information, whole blood, spinal fluid, and fecal specimens were obtained from four American patients (WH, AF, AH, and RM) in a Tokyo hospital and 3 Americans (AR, WD, and CB) hospitalized on Okinawa. Each of these patients showed clinically an abortive non-paralytic poliomyelitis like disease. Fecal material was treated with antibiotics to reduce bacterial contamination and all specimens were inoculated intracerebrally and intramuscularly into 3 to 7 day old suckling mice and also 3 week old mice. All attempts at isolation of virus were negative. Subsequent serological tests showed 4 of the 7 patients (AF, AH, RM, and AR) demonstrating diagnostic titre of complement-fixing antibodies for Japanese B encephalitis. The other three while failing to show a significant rise in complement-fixing antibodies, demonstrated neutralization indices between 1600 and 7900.

RABIES: There were 47 brain specimens submitted to this laboratory for diagnosis of rabies during 1950. This is a marked increase over the 27 specimens received in 1949. Of the 47 submitted, 13 showed Negri bodies and the presence of the virus was confirmed by animal inoculations.

Autopsy material was received from Tokyo Army Hospital of patient (V5-1308 - IM) who succumbed to an encephalitis type infection, etiology unknown (13). This individual had been bitten on the hand by a dog some 30 days previously. Histopathologically, the canine brain tissue was positive for Negri bodies. Subsequently, Pasteur treatment had been initiated and completed. Clinical symptoms developed and progressed into partial paralysis and dyspnea and the patient expired within 24 hours subsequent to hospital admission. Histopathology did not reveal any evidence of Negri bodies on either smear or tissue section preparations. Animal inoculations, using suspension of human brain, produced typical symptoms of rabies in white mice with development of typical Negri bodies.

PORCINE ENCEPHALITIS VIRUS: An encephalitis appeared in a litter of five pigs during July 1950 in Saitama Prefecture. Three of the litter were of normal delivery, one was born moribund, and the other was stillborn 48 hours prior to the expected time of delivery. From the animal born moribund, a neurotropic agent was recovered. This case appearance was reported by the Japanese Animal Industry personnel, and a member of this laboratory was dispatched to the locale to conduct an autopsy. Small sections of brain plug, along with other tissues and organs, were recovered.

Brain tissue of the moribund pig which showed hydrocephalus on autopsy was inoculated intracerebrally into ten 12 to 14 day old albino mice and observed for a 21 day period. One mouse appeared ill on the sixth day showing typical encephalitic symptoms and was sacrificed for further passage. Two other mice were definitely ill on the eighth day and were also sacrificed. Subsequent passages were readily accomplished with an average incubation period of 3 to 4 days. Results of investigation of this agent follow:

(1) Strain designation: V5-1250.

(2) Passage history: Originally isolated in mice (3 of 10 mice initial passage) in July 1950 and subsequently maintained by intracerebral passage in this host.

(3) Experimental Infection: Host range.

Mice - Inoculated with 0.03 ml. intracerebrally with a 10 per cent suspension succumb on the 3rd or 4th day, and the brain titre 10^{-6} to 10^{-7} . Mice weighing 12 to 15 gms. can be infected intranasally, subcutaneously 0.1 ml., intraperitoneally 0.1 ml., and by oral administration (5 to 6 gms. mice with a 10% suspension).

Rabbits - Weighing 2,000 to 2,500 gms. are refractory to the virus when inoculated intracerebrally with 0.1 cc. of a 10 per cent emulsion, based upon negative encephalitic symptoms during a 21 day observation period.

Guinea Pigs - Weighing 250 to 300 gms. inoculated intracerebrally 0.1 ml. with a 10 per cent emulsion, exhibit no manifestations during a 21 day incubation period. Intraperitoneal inoculation of this host with 0.2 cc. of a 10^{-1} emulsion results in a transient elevated temperature ($40^{\circ}\text{C}.$). No further clinical manifestations can be detected during a 14 day observation period.

(4) Cross Neutralization Tests - Neutralization tests conducted with the newly isolated strain, Japanese B encephalitis, and St. Louis encephalitis viruses are shown below:

<u>Immune Sera</u>	<u>Virus</u>	<u>Neutralization Index</u>
V5-1250	V5-1250	32,000
Nakayama		10
St. Louis		10
V5-1250	Nakayama	10
Nakayama		1,600
St. Louis		10
V5-1250	St. Louis	130
Nakayama		10
St. Louis		780

Summary - The general properties of this virus resemble those of the Nakayama strain of Japanese B encephalitis. The pathogenicity of the isolate appears slightly different from that of the Nakayama strain in that subcutaneous intraperitoneal, nasal, and oral infections can be accomplished. Development of symptoms (spastic paralysis) in mice and incubation period are somewhat at variance. There is some suggestion of possession of a common antigen with the virus of St. Louis encephalitis.

Lyophilized specimens were shipped to AMSGS for confirmation of findings. Results of neutralization tests conducted in Washington showed that the "porcine virus was neutralized appreciably by St. Louis, Louping-ill, and Russian spring and summer encephalitis antisera." Those antisera which did not neutralize V5-1250, in addition to Japanese B encephalitis, included West Nile, Colorado Tick and Theilers.

Additional studies are being conducted to establish identity through immunological and serological relationship between this strain and all the well established encephalitides.

INFLUENZA: A total of 442 paired serum specimens representing 888 patients suspected of harboring an upper respiratory infection were received by this laboratory for influenza hemagglutination-inhibition tests (14, 15, 16, 17, 18, 19). Of this group 229 pairs gave negative results, while 213 paired sera (48.2%) showed a diagnostic rise in antibody titre against either influenza A (PR-8), influenza A prime (FM-1), or influenza B (Lee). Crossings appeared in 15 specimens in which a significant rise in antibody titre was demonstrated. A majority of the specimens was received during the months of February and March, which followed the outbreak of the Tokyo American School, Meguro, Japan

Monthly record of tests performed is shown in Table XVIII.

Table XVIII. Influenza Agglutination-Inhibition Tests for 1950

Month	Paired Specimens Tested	Diagnostic for PR-8	FM-1	Lee
January	81	0	0	16
February	106	0	0	50
March	136	0	0	86
April	78	5	3	20
May	18	2	0	1
June	7	0	0	0
July	8	0	0	0
August	12	0	0	0
September	8	1	0	1
October	10	0	0	0
November	19	2	1	0
December	47	8	17	0
Total	442	18	21	174

A few small outbreaks of influenza occurred in American personnel in Japan in early 1950. These consisted of sharply localized groups showing a high attack rate with an acute and short respiratory infection. Investigation of two such epidemics is reported.

American Children in Meguro High School, Tokyo - During the last two weeks of January and extending into early February, a small but sharp outbreak of acute respiratory disease, reaching its peak during the last week in January, occurred among the students of the Tokyo American School, Meguro, Japan. The students ranged in age from 13 to 18.

The Meguro project was started in January with 31 patients being visited for clinical study, collection of throat washings and paired serum specimens for serological confirmation of diagnosis. The disease was mild and self-limiting. Onset was gradual over 24 to 48 hours with the feeling of malaise, headache, aching in the muscles of the eyes, fever, and occasional sore throat, lacrimation, cough and myalgia with temperature ranges from normal to 103°F. with the peak arising in the late afternoon.

Throat washings were collected in phosphate buffer pH 7.8, frozen, returned to the laboratory and stored in CO₂ refrigeration until they could be processed. Antibiotics were added in sufficient amount and inoculated into the amniotic cavity of 11 to 13 day embryonated eggs. Eleven specimens were inoculated and nine second passages accomplished. Following incubation of the second passage, allantoic fluid of six showed presumptive evidence for type "O" human red cell agglutinins. The harvested fluid was sterile and had a hemagglutination-inhibition titre of 1:128 against hyperimmune influenza "B" antisera. On successive passage, the titre was increased to 1:320. Mature roosters were immunized against the new isolate, in addition to Lee, PR-8, and FM-1 strains. The antigenic pattern of the isolate was determined when this antisera was available.

Of acute and convalescent sera tested using the serological agglutination-inhibition test, 14 of 22 paired sera demonstrate a diagnostic antibody rise to influenza B (Lee strain). This group of 22 cases was a fairly uniform and representative one, comprising about 25 per cent of the total cases showing the same clinical entity.

Troops at Camp Haugen - During January and early February, the 32nd Infantry Regiment (7th Division) at Camp Haugen experienced very low rates for common respiratory diseases, with one peak of 140/1000/annum for the week ending 13 January and another of 99 for the week closing 3 February. However, during the week of 24 February, there were 13 admissions from this organization for respiratory infections (rate of 304/1000/annum) of which 12 were considered to be common respiratory diseases and one possibly

influenza. The following week there were 26 admissions, 24 of them suspected of being influenza infections (595/1000/annum). During the week ending 10 March, admissions dropped to 14 and all but one were considered to be influenza. It will be noted that the entire episode lasted but 16 days and half of the 38 cases of influenza occurred within the first six days. There was no particular case distribution by company. All cases appeared to occur quite at random but at no more than a one day interval.

The clinical symptoms were consistent with those of influenza. Two agents isolated from throat washings having agglutination properties for human "O" type cells, have been tentatively identified by hemagglutination-inhibition tests with specific immune sera as the "B" strain of influenza virus. Gross immunogenic studies in fowl for complete differentiation as to type have been accomplished and offers further supportive proof of the antigenic relationship to that of the Lee strain.

Identification of Influenza Strains Isolated - Lyophilized cultures of the I5-Meguro and I5 POOH strains from Camp Haugen were forwarded to the AMSGS for further identification. The following reply was received: "I5-Meguro and I5-POOH strains are identified as influenza B. They are very different from the standard Lee strain and resemble the Warner strain which was isolated in 1948. They are probably identical with Horsfall's so-called "B" prime strain."

RESIDUAL EFFECTS OF JAPANESE B ENCEPHALITIS VACCINATION IN JAPANESE, OKAYAMA, 1950: As has been described in previous annual reports, a project was undertaken by the Virus Commission, Armed Forces Epidemiologic Board, Public Health and Welfare Section, SCAP, Japanese Ministry of Health, the Prefectural Health Department of Okayama, the AMSGS, the National Institute of Health (Japan), and this laboratory. The purpose was to determine the efficacy of JBE vaccine, and ultimately whether or not it might be a feasible method of control of the seasonal and periodic occurrence of the disease in the Japanese. Okayama prefecture was selected based on historic occurrence of the disease in that area. The study was to be conducted in school children from 5-10 years of age, since naturally acquired protection became increasingly more common in older age groups.

In general, standard vaccination was administered to additional numbers of children in 1946, 1948, and 1949 with annual stimulating doses to previously vaccinated individuals each year during the period 1946-1949 inclusive. This resulted ultimately in approximately 55,000 vaccinated children with an equal number of non-vaccinated. The paucity of cases in the area each year did not appear to warrant continuation of the study, and in 1950 vaccine was not administered but an effort was made to follow up the effects in protected and normal groups.

GENERAL ASPECTS OF THE JAPANESE B ENCEPHALITIS EPIDEMIC IN OKAYAMA PREFECTURE IN 1950: The first case of Japanese B encephalitis was reported on 17 July 1950. Following this date, there were only seven additional cases reported until the first of August. Subsequently, a gradual increase in cases occurred up to and including the 13th of August. During the following week a rapid ascent of developing cases was noted with a peak being reached on 19 August. The abatement of the disease was in direct ratio to that of the rise with the last case being reported on 10 September (Fig. 4).

The total number of reported cases was 291 with 85 deaths; seven of these cases were observed in the vaccinated group, one of which succumbed to the infection. The remaining 284 cases were distributed among a total population of all ages numbering 1,592,705 individuals. The morbidity rate among the non-vaccinated population was 0.178 per thousand and the case fatality was 29.6 per cent.

The geographical distribution of cases is shown in Figure 5, indicating a prevalence of disease in the southern half of this prefecture.

Comparison Between the Non-Vaccinated and Vaccinated Individuals - Summarized in Figure 6 are the data on case and death distribution in Okayama Prefecture during the epidemic. This is tabulated according to age groups, location, and groups of vaccinated and non-vaccinated individuals.

Unfortunately, population data at this time still have not been obtained to allow comparison of the older children in the study group, approximately 6,000 of which are

JAPANESE ENCEPHALITIS PATIENTS BY DATE OF ONSET

OKAYAMA PREFECTURE, AS OF 12 SEPTEMBER 1950

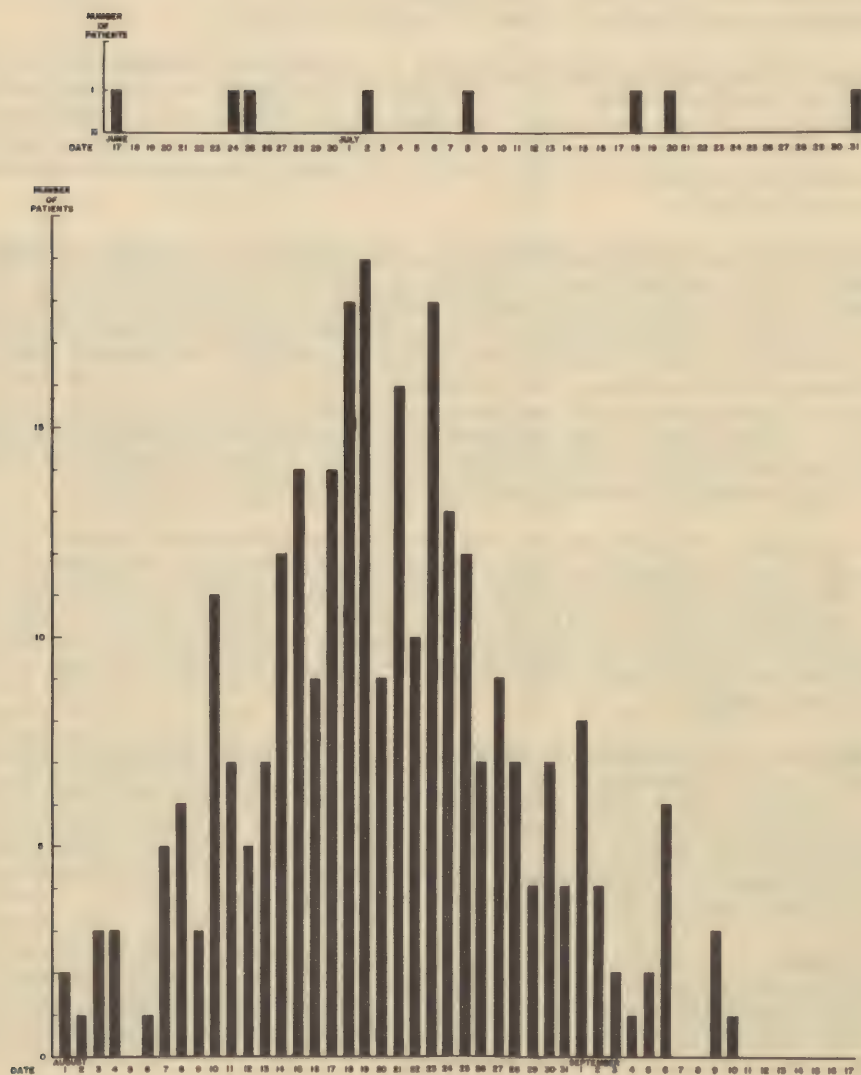


FIGURE 4

JAPANESE B ENCEPHALITIS CASE DISTRIBUTION

OKAYAMA PREFECTURE 1950



FIGURE 5

**CASE DISTRIBUTION ACCORDING TO LOCATION AND AGE GROUPS
AMONG VACCINATED AND UNVACCINATED INDIVIDUALS
IN OKAYAMA PREFECTURE**

CITY & COUNTY	CLASSIFICATION OF AGE VACCINATED AND UNVACCINATED	0-5				6-7				8-10				11-60				OVER 61				TOTAL			
		POPULATION	NO. OF CASES	DEATH	PER-CENT	POPULATION	NO. OF CASES	DEATH	PER-CENT	POPULATION	NO. OF CASES	DEATH	PER-CENT	POPULATION	NO. OF CASES	DEATH	PER-CENT	POPULATION	NO. OF CASES	DEATH	PER-CENT	POPULATION	NO. OF CASES	DEATH	PER-CENT
OKAYAMA-SHI	VACCINATED		0	0		1,115	0			9,514	0			1,739	0			39	0			9,409	0		
	UNVACCINATED	18,997	5			6,442	5			1,943	4	2	50.0	107,082	15	1	6.3	10,575	4	2	50.0	140,579	34	5	14.7
KURASHIKI-SHI	VACCINATED		19	0		595	0			2,100	1			329	0			112	0			3,135	1		
	UNVACCINATED	5,994	5	5	62.5	1,707	0			940	1			34,997	7	3	42.9	3,319	4	3	75.0	46,717	20	11	59.0
TAMANO-SHI	VACCINATED		0	0		280	0			1,893	0			317	0			80	0			2,470	0		
	UNVACCINATED	5,564	3			1,990	1			933	0			29,735	3	1	23.3	2,401	2			39,414	9	1	11.1
KOJIMA-SHI	VACCINATED		3	0		999	0			1,393	0			193	0			49	0			2,495	0		
	UNVACCINATED	4,113	4			720	0			973	1			22,330	1			2,349	0			30,997	5		
TSUYAMA-SHI	VACCINATED		0	0		798	0			1,912	1			406	0			119	0			3,206	1		
	UNVACCINATED	4,075	0			1,740	0			1,408	0			26,999	5	2	33.3	4,050	1	1	100.0	49,395	7	5	42.9
MITSU-GUN	VACCINATED		2	0		632	0			1,749	0			399	0			87	0			2,769	0		
	UNVACCINATED	7,115	4	1	59.0	2,290	2			2,221	0			43,345	5	1	50.0	5,949	7	2	29.5	51,914	19	4	22.2
AKAIWA-GUN	VACCINATED		0	0		212	0			953	0			57	0			21	0			1,193	0		
	UNVACCINATED	6,305	3			2,504	0			2,591	1			39,392	2			5,494	1	1	100.0	56,479	7	1	14.3
WAKE-GUN	VACCINATED		1	0		709	1			1,970	0			997	0			49	0			2,615	1		
	UNVACCINATED	7,995	1			2,692	0			2,947	1			43,790	5	3	50.0	5,953	5			62,937	12	3	29.0
OKU-GUN	VACCINATED		7	0		490	0			1,140	0			302	0			18	0			1,937	0		
	UNVACCINATED	9,997	0			2,409	1	1	100.0	2,499	2			42,309	1	1	100.0	5,977	2			51,123	3	2	39.3
JOTO-GUN	VACCINATED		0	0		800	1			1,244	1	1	100.0	308	0			46	0			2,399	2	1	50.0
	UNVACCINATED	5,339	2	1	50.0	1,904	2	1	50.0	1,746	1			36,291	2			5,992	1			53,114	6	2	25.0
KOJIMA-GUN	VACCINATED		30	0		699	0			2,913	0			623	1			21	0			4,011	1		
	UNVACCINATED	12,239	9	4	44.4	4,365	0			3,540	2			68,082	5	1	20.0	9,575	0			97,820	16	5	31.3
TSUKUBO-GUN	VACCINATED		37	0		1,279	0			2,595	0			499	0			101	0			4,407	0		
	UNVACCINATED	9,144	4			2,142	2			1,949	1	1	100.0	51,477	5	1	20.0	7,579	0			71,191	12	2	16.7
ASAKUCHI-GUN	VACCINATED		0	0		2,209	0			5,599	1			799	0			140	0			6,733	1		
	UNVACCINATED	19,239	12	2	16.7	4,704	3			3,343	5			55,396	11	3	27.3	13,999	0			131,224	31	5	16.1
ODA-GUN	VACCINATED		0	0		1,211	0			2,499	0			310	0			111	0			4,097	0		
	UNVACCINATED	11,794	4	1	25.0	4,099	3	1	33.3	4,941	2	1	50.0	70,276	10	5	50.0	11,399	3	3	100.0	102,163	22	11	50.0
SHITSUKI-GUN	VACCINATED		0	0		0	0			0	0			0	0			0	0			0	0		
	UNVACCINATED	9,319	0			2,303	0			3,315	1			31,727	5	3	50.0	4,611	1			47,475	7	3	42.9
KIBI-GUN	VACCINATED		20	0		412	0			1,367	0			197	0			66	0			2,091	0		
	UNVACCINATED	5,942	1			3,209	3			3,599	2			53,093	12	3	25.0	5,599	8	1	12.5	77,397	26	4	16.4
JOBO-GUN	VACCINATED		0	0		245	0			321	0			5	0			41	0			512	0		
	UNVACCINATED	6,019	0			2,179	0			3,207	0			34,599	0			5,202	1	1	100.0	31,199	1	1	100.0
KAWAKAMI-GUN	VACCINATED		0	0		295	0			329	0			49	0			0	0			624	0		
	UNVACCINATED	5,991	0			2,097	0			3,193	1			31,997	0			5,331	0			46,442	1		
ATETSU-GUN	VACCINATED		0	0		0	0			0	0			0	0			0	0			0	0		
	UNVACCINATED	7,339	0			3,194	0			4,611	0			45,934	0			5,079	0			54,492	0		
MANIWA-GUN	VACCINATED		0	0		6	0			229	0			11	0			22	0			235	0		
	UNVACCINATED	5,499	2	1	50.0	3,931	2			5,105	1	1	100.0	50,494	7	1	14.3	7,612	5	3	50.0	78,207	18	6	33.3
TOMADA-GUN	VACCINATED		0	0		79	0			171	0			29	0			5	0			235	0		
	UNVACCINATED	6,395	1	1	100.0	2,449	0			3,575	0			37,182	2	1	50.0	5,450	2	1	50.0	55,010	5	3	50.0
KATSUTA-GUN	VACCINATED		0	0		0	0			0	0			0	0			0	0			0	0		
	UNVACCINATED	7,649	0			3,259	0			4,994	0			45,002	2	1	50.0	5,903	2	1	50.0	59,399	4	2	50.0
AIDA-GUN	VACCINATED		0	0		0	0			0	0			0	0			0	0			0	0		
	UNVACCINATED	5,379	0			2,317	0			3,281	0			25,341	3			5,031	5	4	80.0	48,349	8	4	50.0
KUME-GUN	VACCINATED		0	0		213	0			693	0			139	0			23	0			1,070	0		
	UNVACCINATED	6,129	0			2,342	0			2,913	0			38,790	1	1	100.0	5,472	5	5	100.0	52,505	6	6	100.0
TOTAL	VACCINATED		119	0		12,939	2			55,909	4	1	25.0	6,639	1			1,075	0			67,580	7	1	14.3
	UNVACCINATED	199,977	63	19	29.4	65,162	24	3	12.5	69,329	25	5	19.2	111,025	111	32	29.5	159,321	50	29	49.7	1,992,705	294	94	29.9

shown in the group age 11-60. Morbidity and mortality data for the younger groups is summarized in Table XIX.

Table XIX. Morbidity and Mortality in Selected Groups

<u>Age Groups</u>	<u>Cases</u>	<u>Deaths</u>	<u>Population</u>
0-5 yrs	0/31	0/4	112/53,872
6-7 yrs	2/24	0/3	9111/34,038
8-10 yrs	4/25	1/5	32090/39751

N.B. Numerator represents vaccinated, denominator unvaccinated children.

A comparison of the expected occurrence of cases in the vaccinated group in terms of incidence in total population, and in terms of incidence in unvaccinated population of the three age groups (Table XX) again suggests, as in previous years, that some protection is afforded by the vaccine, even one year after discontinuance.

Table XX. Expected and Actual Occurrence of Cases in Vaccinated Children

<u>Age Groups</u>	<u>At rate in Total Gp.</u>	<u>At rate in non-vac.</u>	<u>Actual Cases</u>	<u>Protection (?)</u>
0-5	<1	<1	0	Unknown
6-7	6	6	2	66%
8-10	13	21	4	69-81%

NEUTRALIZATION AND COMPLEMENT FIXATION REACTIONS OF HORSES EXPERIMENTALLY INFECTED WITH JAPANESE B ENCEPHALITIS VIRUS: A high incidence of neutralizing antibodies against the virus of Japanese B encephalitis has been observed among horses and human beings in endemic areas of Japan (8, 20, 21). This fact has been considered as due to a wide-spread inapparent infection with the virus and its cumulative antigenic effect, and is supported because the geographical distribution of the neutralizing antibodies coincide with that of the occurrence of cases of Japanese B encephalitis together with serological confirmatory changes which occur during the epidemic season. Further, Kii et al. (22) demonstrated that horses developed positive neutralization indices in which the viremia was confirmed.

Methods and Material - Twenty-four young (one to two years old) horses were obtained from the northern part of Hokkaido (Kitami) for this project. Prior to 1948 this island had been considered as a non-epidemic and non-epizootic area. These animals were procured from an area in Hokkaido where no cases were reported during the 1948 outbreak and were stabled in a suburban area (Yoyogi) of Tokyo.

For inoculation purposes two strains of Japanese B encephalitis were used. The Okinawa strain which was isolated from a fatal human case during 1949, had been adapted by five consecutive passages in mice. The Nakayama strain which is universally employed, is the standard strain of Japanese B virus and has had countless mouse passages since its original isolation in Japan. For the inoculation of horses, suspensions of infected mouse brain of known LD₅₀ titres were used. The infected mouse brain material was titrated prior to inoculation and stored in a dry ice chest until the titration was completed, after which the inoculum was immediately utilized.

Blood samples were collected in the usual manner. Following separation, the sera were stored in dry ice chest remaining there until just prior to testing.

Intracerebral neutralization tests were carried out in mice with 0.2 ml. of undiluted and unheated serum against 0.2 ml. of three or four 10-fold dilutions of mouse brain

suspension infected with the virus of Japanese B encephalitis (Nakayama). The tubes containing serum-virus mixtures were incubated for two hours in a water bath at 37°C. White mice, weighing approximately 8 gms., were then inoculated intracerebrally with 0.03 ml. amounts of each of the serum-virus mixtures. Four or five mice were used per dilution of virus suspension. The inoculated mice were observed for two weeks. A normal rabbit serum known to be negative for Japanese B encephalitis virus was included as a control for each test.

The neutralizing capacity is expressed by the neutralization log index. (The difference in log dilutions of LD₅₀ endpoints of virus-serum mixture containing the serum in question and the negative serum control).

Our technique is similar to that outlined by Casals and Palacios (8) with overnight incubation of serum-antigen-complement mixtures and complement titrations in the presence of antigens at 5°C. The antigens were prepared from the Nakayama strain by the benzol extraction method described by Espana and Hammon (2). A control antigen was prepared from normal mouse brain by the same method. The extent of complement-fixation was expressed numerically, 4 representing complete fixation and 0 as no fixation. Degrees of intermediate or partial fixation is expressed as 3, 2 or 1 and $\frac{1}{2}$. The titre of a serum is the highest original dilution showing 2 $\frac{1}{2}$ or greater fixation.

Changes of Neutralization and Complement-Fixation Titres of Horses from July Through December 1949 (3) - Monthly bleedings were conducted on 24 horses, stabled at Yoyogi in Tokyo for neutralization and complement-fixation tests. Results are shown in Table XXI. None of the horses demonstrated clinical manifestations associated with any encephalitic infection during the period of these observations. Horse No. 22 was autopsied in December, in which neither macroscopic nor microscopic changes were observed.

Changes of Neutralization and Complement-Fixation Titres of Horses Following Experimental Inoculation with Japanese B Encephalitis Virus - For inoculation purposes virus suspensions of known titres were used. Two groups (I and II) of ten horses each (No. 1-10 and 11-20) were inoculated intravenously with 3.0 ml. each of 10^{-6.2} and 10^{-5.2} concentrations, respectively, of mouse brain infection with the Okinawa strain which had a LD₅₀ titre in mice of 10^{-5.7}. Horse No. 21 was injected intravenously with 3.0 ml. of 10^{-7.5} concentration of the virus suspension of the Nakayama strain. The intracerebral titre of this virus suspension was 10^{-8.3} in mice. The remaining two horses (No. 23 and 24) acted as controls and did not receive any inoculation.

On January 18, 1950, just prior to inoculation, blood specimens were drawn with additional serial serum specimens obtained during the ensuing weeks to include February 14. In order to determine the presence of a viremia in the blood specimens, mice were inoculated intracerebrally immediately following bleeding. At no time were we able to demonstrate the presence of circulating virus in the blood stream. The results of neutralization and complement fixation tests on these serum specimens are shown in Table XXII.

The two control animals (No. 23 and 24) remained negative for complement-fixing antibody during the period of observation, as opposed to all animals which received an intravenous inoculation of the virus and subsequently developed complement-fixing antibodies. Five horses had a complement-fixation titre of 1:4 prior to inoculation, and subsequently showed a definite increase following this antigenic stimulation. Of the seven animals in group I, which were negative prior to inoculation, five and two became positive in six and eight days, respectively; while in group II, seven animals (one and six) developed complementing-fixing antibodies in three and six days, respectively. All animals which had received a virus inoculation remained positive during the subsequent four week period of observation. There was one exception; horse No. 3 developed a titre of 1:4 in three days and was negative on the 12th day thereafter.

The highest complement fixation titres which individual animals reached are shown in Table XXIII. As shown, the animals of group II developed a higher titre than those of group I. The average difference in titre is significant.

Table XXI. Changes of Neutralization and Complement-Fixation Titres of Yoyogi Horses Through Epidemic Season

Horse No.	Jul 8		Aug 11		Dates Horses were Bled								Dec 20	
	N*	C**	N	C	Aug 25		Sep 30		Oct 25		Nov 23		N	C
1	0.5	0	0.6	0			4.1	1:16	4.1	1:16	4.3	0	3.7	0
2	0.5	0	0.3	0			3.8	0	3.8	0	4.9	0	3.9	0
3	0.7	0	0.5	0			2.8	0	4.0	0	4.5	0	3.9	0
4	0.3	0	0.7	0			3.4	0	4.2	0	3.4	0	3.7	0
5	1.1	0	0.7	0			3.4	1:8	4.1	1:4	4.0	0	3.9	0
6	0.8	0	1.0	0			3.4	1:4	4.1	1:4	4.4	0	3.7	0
7	0.5	0	0.3	0			3.9	1:8	4.1	1:4	4.5	1:4	4.0	0
8	0.5	0	0.7	0			3.9	1:8	3.6	1:8	4.7	1:8	3.7	1:4
9	0.3	0	0.5	0			2.9	0	3.7	0	4.2	0	4.1	0
10	0.8	0	0.3	0			3.9	1:8	4.5	1:4	5.1	0	4.0	1:4
11	0.9	0	0.3	0			3.9	1:4	4.4	1:4	4.9	0	3.9	0
12	0.7	0	0.7	0			2.0	0	3.4	0	2.9	0	2.7	0
13	1.1	0	0.8	0			3.1	0	4.4	0	4.1	0	3.5	0
14	0.8	0	0.3	0			3.9	1:4	4.1	1:4	4.5	1:4	3.6	1:4
15	0.8	0	0.3	0			4.1	1:4	4.1	0	5.0	0	4.0	0
16	1.6	AC***	0.3	0			4.1	1:8	4.1	1:8	4.8	0	3.5	1:2
17	0.8	0			1.5	0	4.1	1:4	4.6	1:4	4.4	0	3.7	0
18	0.8	0			0.9	0	2.6	0	3.9	0	4.1	0	3.9	0
19	1.0	0			1.3	0	3.3	0	4.1	0	4.3	0	3.9	0
20	2.6	0			1.9	0	3.6	1:8	4.3	1:4	4.8	1:4	3.7	1:4
21	1.0	0			1.1	0	3.7	1:4	4.8	0	4.7	0	4.0	0
22	0.8	0			1.3	0	4.1	1:8	4.5	1:8	5.2	1:4	Autopsied	
23	0.8	0			1.6	0	2.8	0	3.7	0	3.8	0	3.7	0
24	1.2	0			1.3	0	3.8	1:4	3.9	0	4.8	0	4.2	0

* N - Neutralization log index
 ** C - Complement-fixation titre
 *** AC - Anti-complementary

Table XXII. Changes of Neutralization Indices and Complement-Fixation Titres of Yoyogi Horses after Inoculation with JBE Virus

Group	Horse No.	Date Horses were Bled											
		January						February					
		18	21	24	26	28	29	30	1	3	7	9	14
	1	1:4 4.1	1:4 4.7	1:4 4.1	1:16 3.5	1:16 >3.9	1:16 >3.9	1:8 >4.7	1:8 >4.7	1:16 >4.6	1:16 >4.6	1:8 4.1	- 4.5
	2	0 4.3	0 4.1	1:8 3.4	1:16 3.7	1:16 3.7	1:16 >3.9	1:8 >4.8	1:8 >4.8	1:16 >4.6	1:16 >4.6	1:8 3.7	1:8 -
	3	0 4.4	0 4.6	0 3.6	1:4 3.7	- 3.3	- 3.4	0 4.6	0 4.6	0 >4.6	0 >4.6	0 >4.1	0 -
	4	0 2.7	0 2.8	0 3.3	1:8 3.9	1:16 3.5	1:16 3.5	1:16 4.4	1:16 >4.8	1:16 >4.6	1:16 >4.6	1:16 3.9	1:16 3.8
	5	- 4.1	1:4 4.4	1:4 3.9	1:16 3.9	- 3.7	- >3.9	1:8 >4.8	1:8 >4.8	1:16 >4.6	1:8 3.9	1:8 >4.1	1:8 -
	6	0 4.1	0 3.4	1:8 3.6	1:16 3.7	1:32 >3.9	- -	1:32 4.6	1:32 >4.8	1:32 4.4	1:16 >4.1	1:16 >4.1	1:16 4.1
1	7	0 4.4	0 4.3	1:4 3.7	1:8 3.5	- -	1:32 >3.9	1:8 4.6	1:8 4.6	1:8 >4.6	1:8 4.2	- -	1:16 -

Table XXII. Continued

Group	Horse No.	Date Horses Were Bled												
		18	21	January			28	29	30	1	3	February		
				24	26							7	9	14
	8	1:4 4.6	1:4 -	1:16 3.0	1:16 3.9	1:32 3.5	1:32 3.5	1:16 4.4	1:16 4.8	1:32 4.4	1:32 >4.6	1:16 3.7	1:16 4.3	
	9	0 4.1	0 -	1:8 3.7	1:16 3.9	1:16 3.7	1:16 3.7	1:16 4.6	1:16 4.6	1:16 4.0	1:16 >4.6	1:16 >4.1	1:16 4.4	
	10	0 4.1	0 4.4	1:8 3.5	1:16 3.7	- 3.3	- 3.5	1:8 4.6	1:8 4.6	1:16 >4.6	1:16 4.4	1:8 >4.1	1:8 4.5	
	11	0 4.5	0 4.6	1:8 4.1	1:16 4.1	1:16 3.7	1:16 3.3	1:16 4.8	1:32 >4.8	1:32 4.4	1:32 >4.6	1:8 >4.1	1:16 -	
	12	0 2.2	0 2.7	1:4 3.4	1:8 3.7	1:16 3.7	1:16 >3.9	1:16 >4.8	1:16 >4.6	1:16 4.2	1:16 4.2	- 4.1	1:8 4.3	
	13	0 4.4	0 4.8	1:16 3.4	1:16 3.6	1:32 3.7	1:32 >3.9	1:32 4.4	1:32 >4.8	1:32 4.4	1:32 >4.6	1:8 >4.1	1:16 4.5	
	14	1:4 >4.8	1:4 >4.8	1:16 3.9	1:16 3.9	1:64 3.4	1:64 3.5	1:64 >4.8	1:64 >4.8	1:128 4.3	1:128 4.6	1:32 >4.1	1:32 -	
	15	0 4.6	0 4.6	1:16 3.7	1:16 3.6	1:32 >3.9	1:64 >3.9	1:64 >4.8	1:32 >4.8	1:64 4.4	1:64 4.6	1:16 3.9	1:32 4.7	
	16	1:4 4.6	1:4 4.3	1:16 3.7	1:16 3.8	1:64 >3.9	1:64 >3.9	1:64 4.6	1:64 4.8	1:128 >4.6	1:128 >4.6	1:64 >4.1	1:128 5.0	
	17	0 4.4	1:4 -	1:16 >4.1	1:16 >4.1	1:64 >3.9	1:64 >3.9	1:64 >4.8	1:64 >4.8	1:128 4.4	1:128 >4.6	1:32 >4.1	1:64 -	
2	18	0 4.8	0 4.1	1:16 3.7	1:16 3.9	1:32 3.5	1:32 >3.9	1:32 4.4	1:32 >4.8	1:64 >4.6	1:32 >4.6	1:8 4.1	1:16 >4.1	
	19	0 4.3	0 3.6	1:16 3.3	1:16 3.9	1:32 3.5	1:64 3.5	1:32 >4.8	1:64 >4.8	1:64 >4.6	1:64 >4.6	1:16 >4.1	1:32 -	
	20	1:8 4.4	1:8 4.4	1:16 3.7	1:16 3.7	1:64 3.7	1:64 >3.9	1:64 4.6	1:64 >4.8	1:128 >4.6	1:128 >4.6	1:32 3.9	1:32 4.9	
3	21	0 3.7	0 3.7	1:16 4.5	1:16 >4.1	- 3.9	- 3.5	- 3.7	- 4.6	1:64 >4.6	1:64 >4.6	1:16 >4.1	1:32 -	
	23	0 3.6	0 3.7					- 4.6		0 >4.6			0 2.7	
	24	0 3.7	0 4.2					- >4.8		0 >4.6			0 -	

- = Test not conducted

Discussion - In accordance with previous observations (23, 24, 22) horses stabled in Tokyo during the seasonal appearance of Japanese B encephalitis developed neutralizing antibodies during the month of September 1949. Simultaneously, the majority of these horses became positive from negative by complement-fixation tests. It is noteworthy that all of our horses developed positive neutralization indices while 16 of 24 animals (67%) produced complement-fixing antibodies. In contrast to the neutralization log index which

Table XXIII. Frequency Distribution of the Highest Complement-Fixing Titres that Individual Animals Developed

Group	Dilution*						Total
	4	8	16	32	64	128	
I	1	0	6	3	0	0	10
II	0	0	1	2	3	4	10

* Reciprocal of the log

was still at a high level in December 1949, the complement-fixation titre decreased during the same period of time to a negligible level. This confirms the results of a previous study (24). Subsequently, these animals were injected intravenously with given amounts of the virus of Japanese B encephalitis of the Okinawa and Nakayama strain.

Assuming that the mosquito is responsible for the natural infection of horses, we have attempted to estimate the dose which might be inoculated through a mosquito bite. Of necessity there are a number of factors which must be taken into consideration; the titre of the virus which a mosquito harbors in nature, the amount of fluid injected into the victims by the bite of a mosquito, the number of mosquitoes per animal, etc. To the best of our knowledge, no adequate study has been reported which takes into consideration all of the above-mentioned factors. Therefore, we have arbitrarily selected a virus dose having a titre between $10^{1.5}$ and $10^{2.5}$ times the LD_{50} in mice. The virus suspension of the Okinawa strain had an intracerebral titre in mice of 0.03 ml. of $10^{-5.7}$ gms. of the original brain. Therefore, $10^{1.5} LD_{50}$ is equal to 0.03 times $10^{-4.2}$ gms. of the original infected brain, which was equal to 3 ml. of $10^{-6.2}$ concentration. In an identical manner, 3 ml. of $10^{-5.2}$ concentration of the original brain corresponds to the dose of $10^{2.5} LD_{50}$.

There was no remarkable antibody increase in neutralization tests following inoculation with the virus; only few of the animals demonstrated an increase in titre. This may possibly be explained by the fact that most of the animals had almost maximum neutralization log indices prior to inoculation. Two animals (No. 4 and 12) which had relatively low titres prior to inoculation, demonstrated a significant increase of neutralizing antibodies.

In contrast with the results of neutralization tests, all of the animals which received the inoculations with the virus portrayed the development of complement-fixing antibodies. In a previous study (24) one horse which was stabled in Okayama Prefecture and had a positive neutralization index but a negative complement-fixing titre prior to the epidemic season of Japanese B encephalitis of 1947, developed a positive complement-fixing titre during the summer months. Identical results have been obtained in the present study; thus horse No. 20, which had a positive neutralization but a negative complement-fixing titre prior to the epidemic season, showed a complement-fixation titre of 1:8 during September. These data indicate that during the epidemic season, complement-fixing antibodies may develop in immune as well as non-immune horses. This conclusion is further supported by the fact that 86 per cent of 69 normal horses portrayed complement-fixing antibodies during the summer months of 1947 (20). From these observations it is quite evident that immune horses develop complement-fixing antibodies during the seasonal appearance of the disease. This might be a result of inapparent infection. This opinion is supported by data of the present experiment in which immune horses developed complement-fixing antibodies within any encephalitis symptoms following an intravenous inoculation with virus. Although we were unable to demonstrate the presence of a viremia in any of the horses, this does not necessarily mean that it was non-existent. Perhaps this was due to the magnitude of the neutralizing antibodies present.

It is noteworthy that the development of positive complement-fixing antibodies or of the increase in titre occurred rather rapidly (in 3 to 8 days) following inoculation with the virus. In contrast with these results, a relatively slow appearance of complement-fixing antibodies was obtained in horses which were not immune and had

been inoculated with Japanese B encephalitis virus (25). Thus, one horse which received an intracerebral dose of 5 ml. of 10 per cent mouse brain emulsion infected with No. 2869 strain of Japanese B encephalitis virus developed complement-fixing antibodies in eight days, while two other horses which received an identical inoculation of two other strains (No. 2869; Nakayama) of Japanese B encephalitis virus did not develop complement-fixing antibodies during an observation period of nine and seven days, respectively. Similar results were reported by Sabin et al (26) in human beings following inoculation with Japanese B encephalitis vaccine. These facts may be explained by an anamnestic reaction. Further in our experiments this reaction was more remarkable in horses that received a larger, as opposed to a smaller, dose of virus with an earlier appearance of complement-fixing antibodies and the development of higher titres.

Summary - Twenty-four horses which were stabled in Tokyo, of which 22, one and one had negative, equivocal and positive neutralization indices for the virus of Japanese B encephalitis, developed neutralizing antibodies or demonstrated an increase in titre during September 1949, without any apparent evidence of illness.

Fifteen of these animals developed low titre complement-fixing antibodies (1:4 to 1:16 during September 1949. By December all animals had negligible complement-fixing antibody titre (1:2 to 1:4).

On January 18 these animals received an intravenous inoculation with the virus of Japanese B encephalitis and again developed complement-fixing antibodies. This can probably be explained as an anamnestic type reaction. A few animals showed an increase in neutralization titre. A majority of them did not demonstrate this increase probably because of the maximal titre developed prior to artificial inoculation. None of the inoculated animals showed any evidence of illness.

ISOLATIONS OF JAPANESE B ENCEPHALITIS VIRUS FROM NATURALLY INFECTED CULEX TRITAENIORHYNCHUS: The hypothesis of mosquito transmission of Japanese B encephalitis proposed by Mitamura and his co-workers appears the most logical one. One of paramount criteria of this hypothesis is the isolation of virus from mosquitoes as caught in their natural habitat. Mitamura et al (27, 28, 29, 30) isolated Japanese B encephalitis virus from Culex tritaeniorhynchus and Culex pipiens var. pallens. Russian workers have also reported isolations of this virus from Culex of these same species (31).

Still later during the summer of 1948, Kitaoka et al (32) and Fukusumi et al (33) isolated this virus from mosquitoes collected in the Tokyo area. It is noteworthy that Kitaoka and his colleagues obtained the virus from a pool of Anopheles hyrcanus sinensis as well as Culex species.

In view of the fact that most of the isolations recovered by Japanese workers were obtained through the use of blind serial passages and were conducted during epidemic periods, there has been some doubt expressed as to the virus being isolated from the mosquitoes tested. However, it is believed that a number of isolates among those obtained may be considered to be of mosquito origin because of the relatively high morbidity obtained on primary passage in mice.

Because of the aforementioned shortcomings, it was realized that all future attempts to isolate virus from mosquitoes must be conducted under conditions that would more effectively exclude the possibility of laboratory contamination and accidental or natural infection of mice. These attempts would be of particular value because of the fact that other workers had obtained negative results (34, 35, 36).

Following this line of experimentation, Hammon et al (37) succeeded in isolating the virus from C. tritaeniorhynchus under conditions which render the possibility of virus contaminations from other sources extremely unlikely.

Attempted isolations were performed by us from July through September of 1950. Results of isolations performed by the Entomology Department are discussed in that section of this report. Mosquitoes collected daily in the Tokyo area were used as study material. A total of 38 confirmed isolations of Japanese B encephalitis virus have been made from naturally infected mosquitoes which were collected and tested during the Japanese B encephalitis epidemic in this area. All isolates were derived from Culex tritaeniorhynchus which

were naturally infected. The seasonal appearance of infected mosquitoes in the epidemic area and the effect of storage conditions on mosquitoes collected for isolations have been investigated.

Methods - Mosquitoes were collected from bait traps and resting stations located throughout the metropolitan area of Tokyo. In so far as possible mosquitoes were immediately killed, identified, and isolation attempts made from the fresh materials. When large numbers of mosquitoes collected on any given day exceeded the capacity which could be properly handled in the Isolation Section, identified mosquitoes in lots of 100 were sealed in glass ampules and stored at -50°C . until such time as they could be processed. In further reference frozen mosquito suspensions will be distinguished from fresh materials although methods used in handling both groups are identical.

Identified mosquitoes in lots of 100 were placed in a Ten Broeck grinder to which was added 4 cc. of a phosphate buffer diluent to make an emulsion of each lot. The purpose of this diluent was to provide an optimum pH range of 8.1 to 8.3 in addition to acting as a carrier. Antibiotics are added to the buffer to give a final concentration of 1,000 Oxford units of penicillin and 500 micrograms of streptomycin per cubic centimeter. The Ten Broeck tissue grinders were maintained in a chilled state by emersion of the base in cracked ice while the grinding operation was in progress. Following grinding the emulsion was stored for 1 to $1\frac{1}{2}$ hours at $+5^{\circ}\text{C}$., subsequently the material was centrifuged in an "International" refrigerator centrifuge (12 inch angle head) at 1,500 RPM for 10 minutes. Sterility tests for both aerobic and anaerobic organisms were run on each lot of mosquitoes. Five mice from 14 to 16 days of age were inoculated intracerebrally with .03 cc. and intraperitoneally with 0.1 cc. of inoculum from each lot of ground mosquitoes. All inoculated mice were observed twice daily for 14 days after which surviving animals were discarded. Individual mice showing central nervous system symptoms were passed. One blind passage was performed on each lot of those mosquitoes which had not been subjected to storage regardless of absence of symptoms. Blind passages were accomplished on the seventh day subsequent to the initial inoculation without the use of antibiotics.

Results - Fresh Mosquitoes - One hundred and four lots of fresh mosquitoes were inoculated into mice for isolation purposes (Table XXIV). This material represented 61 pools of C. tritaeniorhynchus, 17 Armigeres obturbans, 16 C. pipiens var. pallens, 7 Aedes vexans, and 3 Anopheles hyrcanus sinensis. Thirteen isolates were obtained exclusively from lots of C. tritaeniorhynchus during primary passages in which mice showed encephalitis symptoms. Although 70 blind passages were conducted, none of these progressed to a confirmed isolate.

Table XXIV. Virus Isolates Obtained from Mosquitoes Trapped in Tokyo Area

<u>Species</u>	<u>Materials Inoculated</u>	<u>Lots</u>	<u>Isolates</u>
<u>Culex tritaeniorhynchus</u>	Fresh	61	13
<u>Culex pipiens</u>	Fresh	16	0
<u>Armigeres obturbans</u>	Fresh	17	0
<u>Aedes vexans</u>	Fresh	7	0
<u>Anopheles hyrcanus sinensis</u>	Fresh	3	0
	Total	<u>104</u>	<u>13</u>
<u>Culex tritaeniorhynchus</u>	Frozen	138	25

These isolates were identified as Japanese B encephalitis virus by complement-fixation tests, using antigens prepared from 4th or 5th passage material and standard immune sera of Japanese B encephalitis, St. Louis encephalitis, and Western equine encephalomyelitis (Table XXV).

The number of pools of C. tritaeniorhynchus tested together with confirmed isolates are shown diagrammatically (Figure 7) according to date of mosquito collection.

Table XXV. Identification of Virus Isolates by Complement-Fixation Test

(Fresh Material)

Lab. No.	Source	Collected	Date Collected	Immune Sera		
				JBE	SLE	WEE
M-25	Ueno Park	ABT (horse)	18 Jul	1:32	1:4	0
M-27	Hategaya (Dairy)	ABT (cow)	18 Jul	1:64	1:4	0
M-44	Yoyogi (Stable)	ABT (horse)	25 Jul	1:64	0	0
M-45	Hategaya	ABT (cow)	27 Jul	1:256	1:32	0
M-57	Ueno Park	ABT (pig)	2 Aug	1:32	0	0
M-61	Ueno Park	ABT (horse)	3 Aug	1:8	0	0
M-62	Hategaya	ABT (cow)	3 Aug	1:32	0	0
M-64	Horinouchi Dairy	Resting Station	3 Aug	1:64	0	0
M-65	Yoyogi	ABT (horse)	4 Aug	1:128	0	0
M-68	Hategaya	ABT (cow)	8 Aug	1:64	0	0
M-69	Ueno Park	ABT (horse)	8 Aug	1:16	0	0
M-70	Ueno Park	ABT (cow)	8 Aug	1:16	0	0
M-85	Yoyogi	ABT (horse)	30 Aug	1:128	0	0

ABT - Animal bait trap

Frozen Mosquitoes - One hundred thirty-eight lots of C. tritaeniorhynchus were tested for the presence of Japanese B encephalitis virus. These frozen lots were stored at -50°C. for periods up to eight weeks. Twenty-five isolates were obtained from this series and identified as Japanese B encephalitis virus by complement-fixation tests, as described for fresh mosquitoes (Table XXVI). Figure 7 indicates the number of lots tested and isolates obtained according to date of mosquito collections.

Table XXVI. Identification of Virus Isolates by Complement-Fixation Test

(Frozen Material)

Lab No.	Source	Collected	Date Collected	Date of 1st Inoculation	Immune Sera		
					JBE	SLE	WEE
FM-72	Jikeikai	ABT (pig)	13 Jul	7 Sept.	1:32	0	0
FM-76	Hategaya	ABT (cow)	22 Jul	7 Sept.	1:64	0	0
FM-87	Yoyogi	ABT (horse)	22 Jul	8 Sept.	1:16	0	0
FM-63	Yoyogi	ABT (horse)	28 Jul	6 Sept.	1:128	0	0
FM-55	Yoyogi	ABT (horse)	28 Jul	5 Sept.	1:64	0	0
FM-12	Yoyogi	ABT (horse)	28 Jul	30 Aug.	1:128	0	0
FM-54	Yoyogi	ABT (horse)	4 Aug	5 Sept.	1:64	0	0
FM-41	Yoyogi	ABT (horse)	7 Aug	4 Sept.	1:16	0	0
FM-5	Ueno Park	ABT (cow)	7 Aug	29 Aug.	1:128	0	0
FM-99	Tokyo Hort. School	Resting Sta- tion (pig)	7 Aug	11 Sept.	1:32	0	0
FM-73	Hategaya	ABT (cow)	8 Aug	7 Sept.	1:128	0	0
FM-51	Hategaya	ABT (cow)	8 Aug	5 Sept.	1:128	0	0
FM-94	Yoyogi	ABT (horse)	9 Aug	11 Sept.	1:16	0	0
FM-9	Hategaya	ABT (cow)	10 Aug	29 Aug	1:128	0	0
FM-52	Ueno Park	ABT (horse)	10 Aug	5 Sept.	1:64	0	0
FM-44	Hategaya	ABT (cow)	11 Aug	4 Sept.	1:32	0	0
FM-38	Hategaya	Resting Sta.	11 Aug	1 Sept.	1:128	0	0
FM-31	Yoyogi	ABT (horse)	14 Aug	1 Sept.	1:32	0	0
FM-33	Ueno Park	ABT (horse)	15 Aug	1 Sept.	1:128	0	0
FM-105	Yoyogi	ABT (horse)	16 Aug	22 Sept.	1:16	0	0
FM-109	Yoyogi	ABT (horse)	16 Aug	22 Sept.	1:64	0	0
FM-115	Hategaya	ABT (cow)	17 Aug	25 Sept.	1:64	0	0
FM-117	Ueno Park	ABT	17 Aug	25 Sept.	1:64	0	0
FM-121	Shimotakaido	Cow	18 Aug	25 Sept.	1:64	0	0
FM-116	Yoyogi	ABT (horse)	18 Aug	25 Sept.	1:64	0	0

ABT - Animal bait trap

ISOLATIONS OF JAPANESE B ENCEPHALITIS VIRUS FROM *CULEX TRITAENIORHYNCHUS*

1950

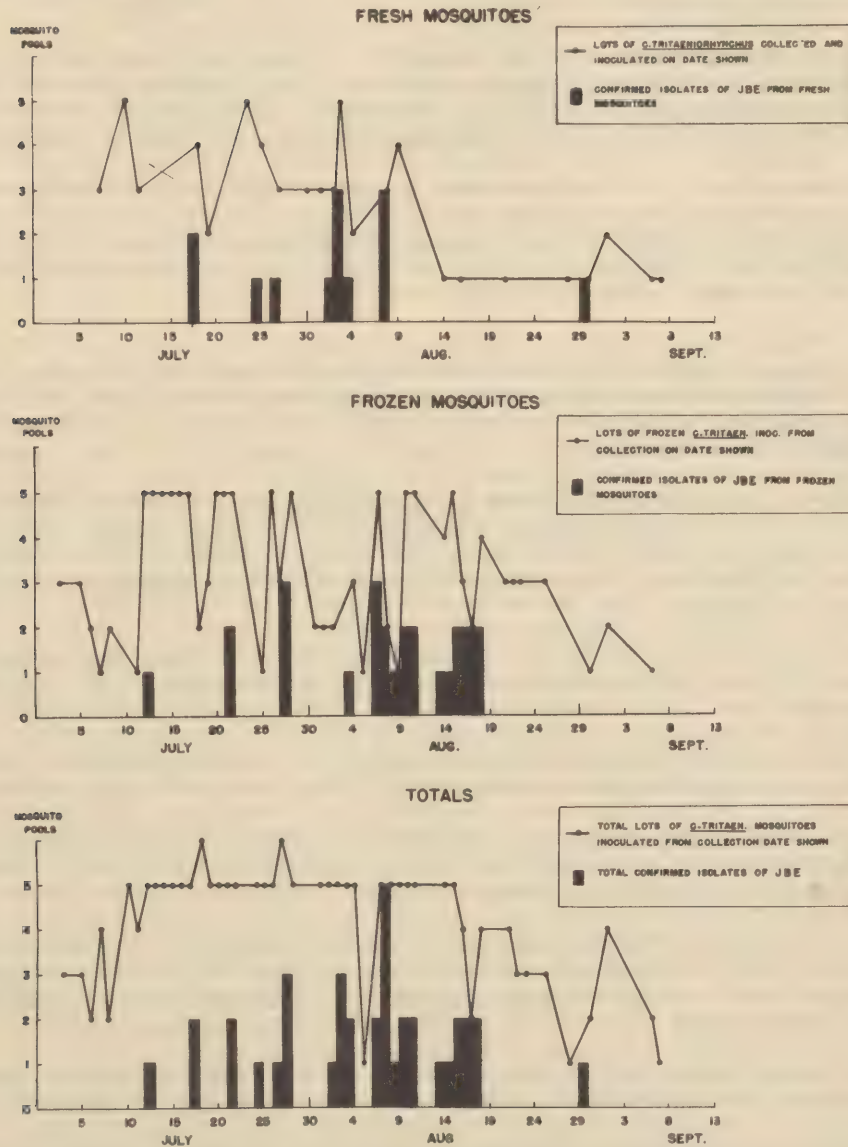


FIGURE 7

Discussion - The mice used for isolation purposes were obtained from Saitama Prefecture, a known area of endemicity for Japanese B encephalitis, which brings up the possibility of a natural infection with the virus of the animals used. Although this possibility cannot be completely excluded, it is felt that these isolates were derived from the mosquitoes tested and not as a source of natural infection of the mice employed. In support of this opinion, it was found that of the 190 mice used for successful isolations, 73 died from encephalitic infection, 75 were sacrificed for further passage after showing encephalitic symptoms, 38 survived without any evidence of illness during the period of observation. The remaining four animals were considered to have died as a result of a traumatic injury. It is inconceivable that this high percentage of encephalitic mice was due to a natural infection.

Further this is supported by the fact that all isolates were obtained in a single species of mosquitoes, C. tritaeniorhynchus, in which 38 of 199 lots gave positive results. Negative results were obtained in 113 series of inoculations which consisted of 43 primary passages with species other than C. tritaeniorhynchus and 70 blind passages.

Identification of the virus was conducted by means of complement-fixation tests, using antigen prepared from mouse passage material of the isolates and known Japanese B encephalitis, St. Louis encephalitis and Western equine encephalomyelitis hyperimmune serum. All isolates gave fixation in the presence of Japanese B immune serum with titres ranging from 1:8 to 1:256.

There was evidence of a common antigenic relationship for the virus of St. Louis encephalitis but always in a low or insignificant titre. Tests in which Western equine encephalomyelitis immune sera were employed against antigens prepared from the isolates were all found to be negative. This preliminary identification indicates that the isolates are the virus of Japanese B encephalitis.

Four species of mosquitoes other than C. tritaeniorhynchus have been tested: Armigeres obturbans (17 lots), Culex pipiens var. pallens (38), Aedes vexans (39), and Anopheles hyrcanus sinensis (3 lots), all of which gave negative results. As previously mentioned, Japanese and Russian workers isolated the virus from C. pipiens var. pallens and Anopheles hyrcanus sinensis. Thus far we have been unable to confirm this finding.

Storage of mosquitoes at -50°C. for periods up to and including eight weeks has little, if any, deterring effects upon isolation of the virus they contain. Thus, 61 lots of C. tritaeniorhynchus which were tested in the fresh state gave 13 isolates (21%) and 25 of 138 lots or 18% of the same species which were used for inoculation purposes after storage were positive (18%) on isolation attempts. Statistically, the difference between the fresh and frozen mosquito pools in percentage of successful isolation is not significant.

In order to have results which were comparable, attempts were made from date of initial collection to maintain a daily inoculation level of five lots of fresh or frozen mosquitoes during the entire period of this experiment. Figure 7 explains in detail these relationships.

Summary - The virus of Japanese B encephalitis was isolated from mosquitoes collected in the Tokyo area. Thirty-eight isolates were obtained from 199 lots of C. tritaeniorhynchus. No isolates were recovered from C. pipiens, var. pallens (16 lots), Armigeres obturbans (17 lots), Aedes vexans (7 lots), or Anopheles hyrcanus sinensis (3 lots).

Storage of mosquitoes at -50 C. for periods up to and including eight weeks appears to have little, if any, deterring effects upon isolation of the virus they contain.

THE ROLE OF MIGRATORY BIRDS IN THE EPIDEMIOLOGY OF JAPANESE B ENCEPHALITIS: During the summer of 1950 serological survey of avian bloods from the Kanto Plain was inaugurated by a representative of the Virus Commission, Armed Forces Epidemiological Board with local assistance of the 406th Medical General Laboratory. The objective was the study of the role of wild birds in the epidemiology of Japanese B encephalitis by determining the presence or absence of neutralizing antibodies in the blood serum of all species found in the endemic area.

Collection of bird sera in the Tokyo area began on 14 July 1950. Frozen serum specimens of various migratory birds were forwarded to Dr. W. McD. Hammon, who reported positive neutralization tests against Japanese B encephalitis virus in 7 of 18 Black Crowned Night Herons, 6 of 10 Cormorants, and 7 of 13 Blue Magpies in an early group of sera examined.

Acting upon the possibility suggested by these serological results, attempts were made to isolate the virus of Japanese B encephalitis from 18 immature Black Crowned Night Herons (Nycticorax nycticorax) and five Cormorants (Phalacrocorax carbo). In each instance one lot of mice were inoculated with a 20 per cent suspension of brain tissue in pH 8.2 phosphate buffer to which a 20 per cent suspension of pooled viscera (spleen, liver, heart, and lungs) utilizing the same diluent previously described. No blind passages were conducted, but 19 serial passages made from mice exhibiting questionable symptomatology failed to produce any actual isolate. These isolation attempts were accomplished after termination of the local epidemic; hence, failure to isolate the virus of Japanese B encephalitis does not necessarily negate the possibility of birds harboring active virus in the epidemic period. The detailed report of laboratory testing of sera will be made by other sources.

The Composition of Some Japanese Bird Populations - While exploring certain valuable epidemiological leads suggested by the avian serological survey, considerable ecological data were obtained. The present report concerns observations obtained from the first thousand birds collected.

Tokyo is located on the alluvial Kanto Plain surrounded by low mountain ranges. Within and about the city are rice paddies and truck farms. Low ridges are covered with mixed deciduous and evergreen woods, including several species of Quercus, Castaneus, Ulmus, Pinus and Cryptomeria. There are many parks, and the city abounds with a verdure of trees and shrubs. All of the ecological niches of the deciduous forests of North America, such as those of Indiana and Kentucky, are evident in the forest about Tokyo and in the low ranges, even though practically all of the existing forest has been hand planted. Within this rich verdant habitat abounding in insects, fruits, nuts, and seeds resides a residual avian fauna.

Japan once had a varied and extensive bird population, claimed to have included 850 species, but widespread use of mist nets and other trapping devices so depleted this fauna that it is making but a slow recovery. Mist netting is now illegal, but the reestablishment of many species will take many years.

Because of low populations, trapping or netting birds was too time consuming for the numbers taken. Most of the species collected were taken by shooting. Twelve gauge and 22 gauge shotguns were used. All birds were bled and examined in the field and representative individuals of each species were retained for positive identification. The area under observation was roughly that within a thirty-five mile radius of the heart of Tokyo. Within this area were most of the habitats found on the Kanto Plain in general, including a 3,000 foot peak, Mt. Takao. The habitats under observation included rice paddies and open cultivated fields, grassy patches, second growth brush land, reeds and coastal marshes, bamboo thickets and wooded farmyards, mesic woods with heavy underbrush, mountain streamsides, and foothill mountain forests.

Nine park areas in the city represented the dense mesic forested areas. These included large oaks, elms, and chesnuts, interspersed with smaller trees, forming a canopy beneath which grew a dense tangle of vine covered shrubs and bamboo. Open areas in the parks were lawns or were covered with a mixture of grass and weeds. In this type of habitat were collected 44 species, mainly arboreal in make-up. Each park supported ponds which were attractive to egrets, cormorants, night herons, kingfishers and ducks. The abundant birds during the summer were the English-sparrow-like Tree Sparrow and the Grey Starling. With the advent of winter the habitat filled with Brown-eared Bulbuls, thrushes, several species of tits, several species of warblers, and a few of the finches.

About the city the cultivated areas presented an open parkland appearance. Here small clumps of trees and dense bamboo plantings were grouped about the farm houses scattered among the fields. Within the farmyard plantings were tree Sparrows, Grey Starlings,

Blue Magpies, Crows, Turtle Doves and in the winter, Bulbuls. Field borders were nearly devoid of birds until several species of buntings and finches entered them for the winter. Grassy areas supported Skylarks and all fields were permeated with Tree Sparrows. The Bull-headed Shrike infiltrated into all of the habitats as it arrived in the winter. Thirty species were collected in these habitats.

On higher ground there were extensive areas of second growth timber interspersed with small cultivated fields. This habitat rich in a variety of woody and fruiting plants with a host of insects was nearly devoid of bird life during the summer. Shrikes, doves and magpies were occasionally seen. In the winter the fauna was characterized by several species of finches and tits. Twenty-four species were taken here.

Highest habitat under observation was that of Mt. Takao and its surrounding hills and streams. This was a heavily treed area of mixed evergreen and deciduous species. It was provided with a very low summer avian fauna, but supported a greater number and variety of birds in the fall and winter. Here were the typically arboreal forms such as the various tits, Japanese Grosbeak, Japanese Hawfinch, Flycatchers, Japanese Jays, Brown-eared Bulbuls, etc. A total of 24 species were collected here.

In the low areas of rice cultivation the avifauna was made up of but few species. Tree Sparrows and Grey Starlings were present all during the period of study. In summer four species of herons and egrets fed along the irrigation ditches, and the Little Egret remained during the winter. Two species of crows were present all of the time. Areas given over to cultivation of reeds supported several species of reed warblers, of which the Eastern Great Reed Warbler was the most abundant summer residents.

Along the extensive tide flats of the shores of Tokyo Bay, a host of shore birds and water fowl gathered during migration and for the winter. Here were the plovers, sandpipers and ducks common to such habitat the work over. At least 25 species of aquatic birds were taken as well as 11 species of reed inhabiting forms from the extensive marshes.

The specialized habitat at the Shin Hama Imperial Duck Refuge was of interest. It provided a protected area of dense bamboo thickets bordering rush and grass filled ponds. It was attractive to the herons and egrets and supported a large colony including seven species. In the winter ducks gathered here in numbers. In summer the bamboo thickets supported Reed Warblers which gave way to Bush Warblers in the winter. Eighteen species of aquatic birds were collected here and 10 species in association with the bamboo thickets.

During the period July 15 to December 15, 1950 approximately 1,000 birds of 95 species were collected. They were taken at random in the various habitats and an attempt was made to collect all individuals of all species that passed before the gun until it was felt that representative samples of sera had been completed. In this way, many of the common species were taken while only small numbers of the less common forms were shot. A few species that were occasionally seen, such as the White-rumped Swift Apus pacificus pacificus (Latham) were not collected. In general, the collections represent the relative abundance and composition of the fauna. Except for isolated colonies of aquatic or shore birds, the summer population was very low, consisting of about 18 summer residents and 26 permanent residents.

Much of Japan including at least the southern half of Honshu lies within the winter range of migrants from the northern islands and from the mainland. For this reason the winter fauna is greater including at least 38 winter residents and the 26 permanent residents. In addition, 11 species were noted passing through the habitat during fall migration. Species in abundance great enough to be collected in numbers in excess of 20 individuals included eight permanent, three summer, and two winter residents. Species slightly less abundant, taken in numbers from 10 to 20 individuals, included seven permanent, one summer, and seven winter residents. Those species occasionally seen, two to nine collected, included 10 permanent, eight summer, 20 winter residents, and 5 migrants. Only one individual was taken of one permanent, six summer, nine winter, and six migrant species.

Sex of all adult and many juvenile birds was determined by dissection or by plumage. The age of the individuals was determined by examining the cranium for degree of calcification. They were listed as birds less than a year old, juvenile, and those older than one year, adult.

Apparently, a random sample of the birds that were seen while walking through the various habitats did not give a true picture of the sex ratios; consistently, more males were taken than females, probably resulting from the more aggressive nature of the males and their more conspicuous coloration. The overall sex ratio was as high as 2.03 males per one female in July to as low as 1.05 males per one female in November, as shown in Table XXVII. The method of sampling the population gave a much better picture of the ratio of juvenile to adult birds from month to month. Peak numbers of juveniles appeared in August with 4.4 young per adult, and gradually this ratio decreased until there were 1:1 young per adult in December (Table XXVII). This decrease probably resulted from actual losses among the young birds as well as the increased calcification of the cranium which rendered identification of young birds difficult.

Table XXVII. The Average Sex and Age Ratios of All Species of Birds Collected on the Kanto Plain between July the 14th and December 15, 1950

<u>Month</u>	<u>Male</u>	<u>Female</u>	<u>Juvenile</u>	<u>Adult</u>	<u>No. of Birds</u>
July	2.03	1	1	1.1	111
August	1.5	1	4.4	1	270
September	1.7	1	3.0	1	152
October	1.6	1	1.7	1	164
November	1.05	1	1.3	1	193
December	1.05	1	1.1	1	63

Thirteen species were taken in excess of 20 individuals. These are listed in Table XXVIII) with the sex and age ratios given. The resident status is also indicated. Within the city the most abundant permanent residents were the Tree Sparrow and Grey Starling, summer resident the House Swallow, and winter resident the Brown-eared Bulbul. Table XXIX gives the same data for 15 of the less abundant species. The Little Egret was common in rice paddies but was so wary that it was difficult to take. The two common crows, Jungle (Corvus leuallantii japonensis Bonaparte) and Carrion (Corvus corone orientalis Eversmann), were as abundant as the Blue Magpie, but in typical crow fashion avoided man effectively. Among those species collected in excess of 20 individuals, the sex ratio was 1.7 males to one female and 2.6 young to one adult. Among those species, 10 to 19 of which were collected, the sex ratio was 1.3:1 and the juvenile-adult ratio was 1.8:1.

Forty-one species were encountered occasionally or were localized in their distribution. These are listed according to their resident status in Table XXX with the juvenile-adult ratio given to indicate the numbers collected. Among these species taken in small numbers, the sex ratio was 1.2 males to one female, and the juvenile-adult ratio average was 1.7:1. An additional 22 species were collected only once.

During the six month period of collections, the common species showed some interesting changes in juvenile-adult ratios. In July the flocks of Grey Starlings appeared to be composed of about half adults and half juveniles. During August this ratio increased to seven juveniles to one adult. From then through November the number of juveniles slowly decreased until by December the cranial characters no longer clearly distinguished young from old. The Tree Sparrow showed much the same pattern with peak numbers of young in the flocks in September. The Blue Magpie, another late nester, also had greatest concentrations of young in September. This species travels within its range in family groups that later in the winter band together into small flocks of 10 to 20 individuals. The House Swallow migrated during August and September and the August flocks were made up of five young to one adult. The Eastern Turtle Dove nested until October and the young made up 85 per cent of the population at that time. This resulted from migration of adults' leaving small flocks of young in the habitat. Winter flocks appeared to be made up of equal numbers of young and old birds. The Bull-headed Shrike was a permanent resident, but its winter population density was approximately one hundred times as great as that

Table XXVIII. Species of Birds Commonly Seen in the Vicinity of Tokyo, Japan
and Collected in Numbers in Excess of Twenty Individuals

<u>Species</u>	<u>Resident *Status</u>	<u>Male-Female Ratio</u>	<u>Juvenile-Adult Ratio</u>	<u>Total Taken</u>
Grey Starling (Mukidori) <u>Spodiospar cineracea</u> (T)	P	19-8	97-25	122
Tree Sparrow (Suzume) <u>Passer montanus saturatus</u> Stejneger	P	16-3	89-5	94
Black-crowned Night Heron (Goisagi) <u>Nycticorax nycticorax nycticorax</u> (L)	P	6-8	36-7	43
Japanese Blue Magpie (Onaga) <u>Cyanopica cyanus japonica</u> Parrot	P	9-3	29-13	42
Eastern House Swallow (Tsubame) <u>Hirundo rustica gutturalis</u> Scopoli	S	4-6	31-7	38
Great Eastern Reed Warbler (O-yoshikiri) <u>Acrocephalus arundinaceus orientalis</u> (T & S)	S	23-12	1-34	35
Eastern Turtle Dove (Kizibato) <u>Streptopelia orientalis orientalis</u> (Latham)	P	7-7	19-15	34
Great Tit (Shijukara) <u>Parus major minor</u> T & S	P	5-4	14-8	22
Japanese Teal (Kogamo) <u>Anas crecca crecca</u> L.	P	13-11	16-15	31
Bull-headed Shrike (Mozu) <u>Lanius bucephalus bucephalus</u> T & S	W	9-9	7-14	21
Plumed Egret (Chu-sagi) <u>Egretta intermedia intermedia</u> Wagler	S	5-1	16-4	20
Skylark (Hibari) <u>Alauda arvensis</u> L.	P	14-5	4-16	20
Brown-eared Bulbul (Hiyodori) <u>Ixos amaurotis amaurotis</u> (T)	W	11-6	10-10	20
Total		141-84	459-173	642

* Permanent - P; Summer - S; Winter - W.

Table XXIX. Species of Birds Slightly Less Abundant in the Tokyo Area
Than Those Listed in Table XXVIII; 10 to 19 Specimens Collected

<u>Species</u>	<u>Resident *Status</u>	<u>Male-Female Ratio</u>	<u>Juvenile-Adult Ratio</u>	<u>Total Taken</u>
Jungle and Carrion Crow (Karasu) <u>Corvus</u> spp.	P	7-2	12-7	19
Japanese Cormorant (Oo) <u>Phalacrocorax carbo</u> <u>hanedae</u> Kuroda	P	6-2	10-7	17
Pied Wagtail (Haku-sekirei) <u>Motacilla alba lugens</u> Gloger	W	6-10	5-12	17
Little Egret (Kosagi) <u>Egretta garzetta garzetta</u> (L)	P	6-0	12-4	16
Domestic Pigeon (Hato) <u>Columba livia</u> L.	P	5-2	10-6	16
Meadow Bunting (Hojiro) <u>Emberiza cioides ciopsis</u> Bonaparte	P	4-1	13-2	15
Ringed Plover (Chidori) <u>Charadrius</u> spp.	W	3-6	14-0	14
Japanese Jay (Kakesu) <u>Garrulus glandarius japonicus</u> T & S	W	10-3	8-5	13
Japanese Bunting (Aoji) <u>Emberiza spodocephala personata</u> T.	W	4-4	9-3	12
House Martin (Iwa-tsubame) <u>Delicon urbica dasypus</u> (Bonaparte)	S	1-2	8-3	11
Green Finch (Ko-kawarahiwa) <u>Chloris sinica minor</u> (T & S)	P	6-2	9-2	11
Pintail (Onaga-gamo) <u>Anas acuta acuta</u> L.	W	1-10	7-4	11
Mallard (Ma-gamo) <u>Anas platyrhynchos platyrhynchos</u> L.	W	5-6	6-5	11
Willow Warbler (Mushikui) <u>Phylloscopus</u> spp.	W	8-0	8-3	11
Black-tailed Gull (Umi-neko) <u>Larus crassirostris</u> Vieillot	P	2-6	2-8	10
Total		74-56	133-71	204

* See footnote to Table XXVIII.

Table XXV. Species Distribution of Birds According to Resident Status

<u>Species</u>	<u>Resident *Status</u>	<u>Juvenile-Adult Ratio</u>	<u>Number of Birds</u>
Bamboo Pheasant (Ko-jukei) <u>Bambusicola thoracica thoracica</u> (T)	P	9:0	9
Narcissus Flycatcher (Kibitaki) <u>Muscicapula narcissina narcissina</u> (T)	M	3:6	9
Japanese Kingfisher (Kawasemi) <u>Alcedo atthis bengalensis</u> Gmelin	P	7:1	8
Chinese Little Grebe (Kaitsuburi) <u>Podiceps ruficollis poggei</u> (Reichenow)	S	7:1	8
Common Sandpiper (Iso-shigi) <u>Tringa hypoleucos</u> L.	S	6:2	8
Grey Wagtail (Kisekirei) <u>Motacilla cinerea caspica</u> (Gmelin)	P	4:4	8
Daurian Redstart (Jobitaki) <u>Phoenicurus auroreus auroreus</u> (Passas)	W	0:8	8
Long-Tailed Tit (Enaga) <u>Aegithalos caudatus trivirgatus</u> (T & S)	W	3:5	8
Wandering Tattler (Kiashishigi) <u>Tringa incana brevipes</u> (Vieillot)	M	5:1	6
Spot-billed Duck (Karu-gamo) <u>Anas poecilorhyncha zonorhynchia</u> Swinhoe	P	5:0	5
Tufted Duck (Kinkurohaji) <u>Aythya fuligula</u> (L)	W	4:1	5
Rustic Bunting (Kashiradaka) <u>Emberiza rustica latifascia</u> Portenko	W	3:2	5
Japanese Wagtail (Seguro-sekirei) <u>Motacilla grandis</u> Sharpe	W	0:5	5
Brambling (Atori) <u>Fringilla montifringilla</u> L.	W	3:2	5
Chinese Little Bittern (Yoshigoi) <u>Ixobrychus sinensis sinensis</u> (Gmelin)	S	4:0	4
Black-eared Kite (Tobi) <u>Milvus migrans lineatus</u> (Gray)	P	4:0	4
Broad-billed Sandpiper (Kiriai) <u>Limicola falcinellus sibirica</u> Dresser	W	4:0	4
Pallas Dipper (Kawa-garasu) <u>Cinclus pallasii hondonensis</u> Momiyama	P	1:3	4
White-eye (Mejiro) <u>Zosterops palpebrusa japonica</u> T & S	W	2:2	4
Falcated Teal (Yoshi-gamo) <u>Anas falcata Georgi</u>	W	4:0	4

Table XXX. Continued

<u>Species</u>	<u>Resident *Status</u>	<u>Juvenile-Adult Ratio</u>	<u>Number of Birds</u>
Brown Thrush (Akahara) <u>Turdus chrysolaus chrysolaus</u> T.	W	4:0	4
Garganey (Shima-aji) <u>Anas querquedula</u> L.	W	4:0	4
Black-headed Gull (Yuri-kamome) <u>Larus ridibundus sibiricus</u> Buturlin	W	3:1	4
Amur Green Heron (Sasa-goi) <u>Eutorides striatus amurensis</u> (Schrenck)	S	3:0	3
Brown Flycatcher (Ko-same-bitaka) <u>Alseonax latirostris latirostris</u> (Raffles)	S	3:0	3
Japanese Grosbeak (Ikaru) <u>Eophona personata personata</u> (T & S)	W	1:2	3
Varied Tit (Yama-gare) <u>Parus varius varius</u> T & S	W	1:2	3
Whimbrel (Chusyaku-shigi) <u>Numenius phaeopus variegatus</u> (Scopuli)	M	3:0	3
Stint (Tonen) <u>Calidris</u> spp.	M	3:0	3
Fan-tailed Warbler (Sekka) <u>Cisticola juncides brunniceps</u> (T & S)	P	2:1	3
Japanese Goatsucker (Yotaka) <u>Caprimulgus indicus jotaka</u> T & S	S	0:3	3
Pygmy Woodpecker (Ku-gera) <u>Dryobates kizuki nippon</u> (Kuroda)	P	2:1	3
Wigeon (Hidori-gamo) <u>Anas penelope</u> L.	W	1:2	3
Dusky Thrush (Tsugumi) <u>Turdus naumanni eunomus</u> T.	W	1:2	3
Water Pipit (Ta-hibari) <u>Anthus spinoletta japonicus</u> T & S	W	1:2	3
Thick-billed Shrike (Chigu-mozu) <u>Lanius tigrinus Drapiez</u>	S	1:1	2
Indian Moorhen (Ban) <u>Gallinula chloropus indica</u> Blyth	P	0:2	2
Spotted Flycatcher (Ezo-hitaki) <u>Hemichelidon griseisticta</u> Swinhoe	M	2:0	2
Green Woodpecker (Ao-gera) <u>Picus awokera awokera</u> T.	P	1:1	2

Table XXV. Continued

<u>Species</u>	<u>Resident *Status</u>	<u>Juvenile-Adult Ratio</u>	<u>Number of Birds</u>
Grey Plover (Daizen) <u>Squatarola squatarola</u> (L)	M	2:0	2
Kentish Plover (Shiro-chidori) <u>Charadrius alexandrinus dealbatus</u> (Swinhoe)	W	2:0	2
Japanese Hawfinch (Shime) <u>Coccothraustes coccothraustes japonicus</u> T & S	W	2:0	2
Goldcrest (Kiku-itadaki) <u>Regulus regulus japonensis</u> Blakiston	W	0:2	2
Large Egret (Dai-sagi) <u>Egretta alba alba</u> L.	S	2:0	2

* Permanent - P; Winter - W; Summer - S; Migrant - M.

Table XXXI. The Changes in Juvenile Adult Ratios in Eight Common Species of Birds as Indicated by Specimens Collected at Random in the Tokyo Area

<u>Species</u>	<u>Jul</u>	<u>Aug</u>	<u>Sept</u>	<u>Oct</u>	<u>Nov</u>	<u>Dec</u>	<u>Average</u>
Grey Starling	1:1	7:1	3:1	2.3:1	2:1	1:4	4:1
Tree Sparrow	12:1	18:1	22:1	14:1	8:0	2.5:1	10:1
Blue Magpie	1:1	2.5:1	5:0	1.5:1	2.5:1	-	2.2:1
House Swallow	1:1	5:1	-	-	-	-	4:1
Eastern Turtle Dove	-	1:1.4	2.5:1	6:1	1:1	0:1	1.2:1
Bull-headed Shrike	-	2:1	1:1.2	1:5	0:1	0:2	1:2
Skylark	-	0:3	1:6	1:1.6	0:2	-	1:4
Brown-eared Bulbul	-	-	-	1:2	6:1	0:1	1:1

of the summer. During August there were twice as many young as adults present. By October the influx of birds from the north had changed this until there were five times as many adults as young. Apparently both the Skylark and the Bull-headed Shrike develop adult cranial characters quickly, for it would seem impossible for a population to maintain itself with such a small proportion of young. In September the Skylark population appeared to have six times as many adults as young, and at no time was the situation reversed. Further study of these two species may throw light on this discrepancy. When the Brown-eared Bulbul arrived in October, its population was already fairly well balanced and the three month average from October through December showed a 1:1 ratio between young and old individuals. Table XXXI illustrates this data.

Summary - During the period July 15 to December 15, 1950 in a study relative to the relationship between wild birds and Japanese B encephalitis, approximately 1,000 birds of 95 species were collected within a 35 mile radius of the center of Tokyo.

The avian fauna included about 45 species during the summer and 65 species during the winter. The winter abundance of birds was much greater than that of the summer.

The sex ratio of the species collected showed a predominance of males. The data was probably distorted by the greater ease with which males could be located.

The population appeared to be made up of 80 per cent juvenile and 20 per cent adult birds in August. This gradually leveled off to 50 per cent young and 50 per cent old birds by the middle of December.

The sex ratios and juvenile-adults ratios of some of the common species are given and the habitat relationships are discussed.

A CASE REPORT OF HUMAN RABIES: Rabies in man is a disease of great antiquity. Because of the dreaded clinical reaction and the fact that once rabic symptoms develop, the disease is uniformly fatal, it is always one of clinical interest.

This disease is unique in two respects; first, because time of exposure is usually well established and secondly, because the incubation period is often sufficiently prolonged so that an active immunity can be produced prior to the establishment of the virus in the vital centers of the central nervous system.

This communication is a report of an unusual case of rabies in that the virus was isolated and identified both from the dog inflicting the bite and the patient dying of rabies and this despite the apparent satisfactory vaccine treatment of the patient starting nine days after the bite was inflicted.

Case History - On the evening of 18 June 1950 a stray dog attacked a 31 year old colored male inflicting a bite of the right thumb. The wound was not treated until the following day when it was cleaned and dressed. The dog was captured, sacrificed, and the head was forwarded to the 406th Medical General Laboratory. Sections and smears of the dog's brain showed the presence of Negri bodies and the diagnosis of rabies was subsequently confirmed by mouse inoculation.

On 27 June 1950, 9 days after the above biting incident, the usual course of anti-rabies vaccine (Semple type) therapy was started. The patient received 14 daily injections, the course ending on 11 July. At no time did he exhibit any allergic or untoward reaction to the vaccine.

On 16 July 1950, 5 days after completion of the above series of inoculations, the patient developed pain and swelling of the right thumb at the site of the dog bite and extending up the hand and arm. There was tingling and numbness of the right hand and fingers and he developed a severe headache. The symptoms persisted during the 24 hours prior to hospitalization on the evening of 17 July 1950.

Severe frontal and right temporal headache and the pain in the right thumb and hand were the chief complaints on admission. Physical examination at this time showed very little. There was slight swelling and tenderness in the right thumb, decreased pain sensation in the right arm, and pain on movement of the right shoulder. There was no nuchal rigidity and no other neurological abnormality. Roentgenograms showed no evidence of bone pathology or arthritic changes. Lung fields were clear and the heart and mediastinum were not unusual.

Course - Temperature showed a gradual rise from 101.2°F. on admission to 104.2°F on 19 July and a drop to 101°F. on 20 July, the day of death. He remained generally well oriented but had intermittent spells of clouding of the sensorium. He developed somewhat bizarre neurological findings including small fixed pupils, slight nuchal rigidity, and decreased abdominal reflexes on the right and exaggerated abdominal reflexes on the left. Knee jerks were exaggerated on the right absent on the left. There was definite weakness of the right arm with absent reflexes while the left showed neither weakness nor abnormal reflexes. On 19 July there was definite flaccid paralysis of the right arm and the patient had considerable difficulty in swallowing. There was pooling of saliva in the fauces. On 20 July right facial weakness was noted. At 1055 hours he suddenly stopped breathing. Artificial respiration and immediate tracheotomy were performed. The patient was pronounced dead shortly before noon 20 July, approximately four days after the onset of clinical symptoms, 19 days after a full course of anti-rabies vaccination was started and 31 days after being bitten on the right thumb by a rabid dog.

Clinical Laboratory Data - Urinalyses and a cardiolipin flocculation test were negative. On 18 July, white blood cell count was 12,400. On 19 July, WBC were 7,900 with the differential count showing 73% polymorphonuclear leukocytes, 19% lymphocytes, 7% monocytes, and 1% eosinophilic leukocytes. Hemoglobin was 15.8 grams. Spinal fluid contained 320 WBC per cmm., 77% of which were lymphocytes and 23% neutrophilic leukocytes.

Autopsy Findings - Autopsy was performed three hours after death. Except for a partly crusted, healing, linear, superficial, 1.3 x 0.3 cm. wound on the palmar surface of the right thumb at the inter-phalangeal crevice, the only macroscopic abnormal changes found were in the brain. Spinal fluid was increased in amount and slightly cloudy. Vascular markings over the surface and on the cut sections of the brain were unusually prominent. The ventricles showed no gross change. There was no evidence of cerebellar pressure cone formation, of cerebral hemorrhage or of tumor. The brain weighed 1390 grams after removal of the specimens for virus study. It was noted that the right upper extremity showed no discoloration, edema, or swelling and that the epitochlear nodes were not palpable. The right radial nerve likewise showed no gross changes. Tissues for virus isolation was removed from the right parietal lobe and the left hippocampus employing the usual aseptic precautions.

Microscopic examination showed edema of the leptomeninges accompanied by a mild, diffuse infiltration of mononuclear cells. Sections of the brain showed a diffuse encephalitis with the following distribution: changes in the cerebral and cerebellar cortex were agonal in type and non-specific, in the basal ganglia specific changes were mild, in the hippocampus, hypothalamus, pons, and medulla progressively severe and intense. These changes consisted of varying degrees of perivascular cuffing, inflammatory and glial nodular infiltrations, focal areas of degeneration of the ground substance and of neurones, satellitosis and neuronophagia. Of the many nuclei involved, changes were most prominent in the substantia nigra and the inferior olivary nuclei. Special stains including Giemsa's, Sellers' and van Gieson's were used in a search for Negri bodies. Known positive sections including sections of mouse brain infected with the tissue from this case were used as controls. Although the controls were consistently positive, no Negri bodies could be found in the sections of the patient's brain.

Sections of the right radial nerve from three different levels showed only a single small area of hemorrhage associated with mild, non-specific inflammation. Sections of the wound on the right thumb showed a chronic, non-specific wound with hyperkeratosis, acanthosis, diffuse scarring of the dermis, and a superficial and deep, diffuse and focal infiltration of inflammatory cells, predominantly mononuclear. These were accumulated about blood vessels and nerves which showed obliterative endarteritis and perineural fibrosis.

Laboratory Diagnosis - Following receipt of central nervous system tissues in this laboratory, the hippocampus was removed. A suspension was made and injected intracerebrally into 10 mice two weeks of age. Within eight days all the inoculated mice developed typical central nervous system symptoms and died during the following 48 hours. Microscopic examination of smear impressions made from the hippocampus and stained according to Sellers' method (40) were positive for Negri bodies. Paraffin sections stained by van Gieson's method were also positive for Negri bodies.

Harvested brain tissues of moribund mice inoculated intracerebrally into rabbits and guinea pigs produced typical paralytic rabies in rabbits with an incubation period of 12 days. The clinical manifestations exhibited in guinea pigs after a 10 day incubation period showed the animals to be ataxic from a flaccid paraplegia. A rise in temperature was observed in both guinea pigs and rabbits within 8 to 10 days after the inoculation of virus.

Intracerebral cross neutralization tests performed on the isolate against known immune serum prepared in rabbits against fixed rabies and Japanese B encephalitis viruses, demonstrated that fixed rabies immune serum neutralized its homologous virus and the newly isolated strain. There was no evidence of cross neutralization with the heterologous virus of Japanese B encephalitis.

Discussion - Despite the application of anti-rabies treatment, this individual succumbed to an encephalitis consistent with that of rabies. Animal inoculations, histopathological and clinical findings confirmed this diagnosis. Although the incubation period was less than 30 days, this is not unusual in that this occurs in one-third of treated and reported cases (41).

Numerous authors have recorded the presence of Negri inclusion bodies in human material, although these are not so commonly present as they are in certain animals. Busson (42) stated that Negri bodies were absent in over 58 per cent of those who developed rabies despite anti-rabies treatment. While we were unable to demonstrate Negri bodies in the human brain, they were readily observed in mice inoculated with suitable autopsy tissues.

Hyperimmune anti-rabies serum prepared in rabbits with fixed rabies virus exerted a definite neutralizing effect against the isolated agent confirming the diagnosis of rabies.

Summary - A soldier bitten on the thumb by a rabid dog was given a complete prophylactic anti-rabic vaccination series, starting 9 days subsequent to exposure and completed 5 days prior to the onset of clinical symptoms. This individual expired 31 days after being bitten. Virus isolation and histologic examination of both the dog's brain and the patient's brain confirmed the diagnosis of rabies. Negri bodies could be found in preparations of the dog's brain and of mice brain injected with both dog and the patient's brain but not in preparations from the patient's brain.

FUJI-SUSONO SURVEY FOR SCRUB TYPHUS: During the latter part of April, this department conducted a field survey of the Fuji-Susono maneuver area for the purpose of collecting vectors and reservoir hosts of tsutsugamushi disease. Previous epidemiological study (X) pointed to the fact that several mild and inapparent cases occurred in military personnel in 1948 on maneuvers at the foot of Mt. Fuji arose from exposure to vectors of the disease in this area.

Frequent evidence of small burrowing rodents was seen in the camp sites along the banks of ravines. Trap lines were set in likely places and a high percentage of catches were made, using frankfurters, peanuts, and cheese as bait. Twelve voles (Microtus montebelli) known to act as the principal reservoir host of tsutsugamushi disease of north-western Honshu, were captured. Twenty-one eastern field mice (Aroderus speciosus speciosus Temmin) and 20 northern mole shrews (Urotrichus talpoides hondonis Thomas) were captured and all species yielded numerous mites as well as lice and a few fleas. Combing of the voles yielded 611 ectoparasites which were identified as Trombicula species as shown in Table XXXII.

Table XXXII. Source and Identify of Mites Collected

No. of Mites	Yamanaka Area			Subashiri Area		Total
	<u>Apodemus</u>	<u>Microtus</u>	<u>Urotrichus</u>	<u>Apodemus</u>	<u>Urotrichus</u>	
	21	11	20	7	4	
<u>T. palpalis</u>	54	20	1	0	0	75
<u>T. intermedia</u>	2	21	2	0	0	25
<u>T. japonica</u>	0	0	1	0	1	2
<u>T. fuji</u>	0	0	0	67	0	67
<u>Gahrillepia</u> sp.	0	5	0	0	1	6
<u>Trombicula</u> for Rickettsial Isolation	211	170	0	55	0	436
Total	267	216	4	122	2	611

Splenic and liver tissue suspensions from each animal were inoculated into white mice for attempted recovery of rickettsiae. Pools of mites (436 unidentified) recovered from the rodents were also inoculated. Two isolates were obtained from splenic emulsions of two Microtus trapped near the entrance to Camp McNair. The third was from an Apodemus near the same locality.

During the course of these rickettsial isolation attempts, a spirochete was isolated in guinea pigs and identified as Leptospira hebdomadis. Silver preparations of kidney tissue revealed the presence of Leptospira in these small rodents. This species is commonly found in cases of seven day fever in Japan.

Small Pox - During the year 1950 three specimens were received from American cases suspected of small pox infection. The first (V5-1196, SRB) was a year old, female dependent of a Korean missionary, one of a number of evacuees from Korea, hospitalized at the Osaka Army Hospital. The second (V5-5571, CWD) was a hospital corpsman on board the hospital ship USS Consolation, which was anchored off the Korean port of Wonsan at the time. The corpsman had been exposed while on duty with marine ground troops in the Seoul-Inchon area. CWD (V5-5571) was the only case of the three which terminated fatally.

In each instance the specimen received was pustular material dessicated on a sterile swab inserted in a test tube containing anhydrous phosphorus pentoxide in the manner recommended by Smadel (43). Upon receipt, the sterile swabs were washed with 2 cc. physiological saline solution containing 1,000 units penicillin and 500 micrograms of streptomycin per cubic centimeter. One tenth cc. amounts of the solution were inoculated on the dropped chorio-allantoic membrane of 10 day embryonated eggs. After four days of incubation the eggs were harvested, examined, and part of the chorio-allantoic membrane was fixed in Zenker's solution and forwarded to the Pathology Department for tissue sectioning. Histopathologic examination revealed Guarnieri bodies of Variola virus in each of the three isolates obtained. Dermal inoculation into rabbits with the infected chorio-allantoic membrane produced a vesicular lesion histologically typical of small pox, including typical inclusion bodies.

HERPES: One specimen of spinal fluid (V5-3280, VC) was received from Tokyo Army Hospital from a patient with the skin lesions of herpes and also showing symptoms indicative of a mild encephalitis. Three week old Saitama mice were inoculated intracerebrally and observed. Two serial passages were made of brain material from mice showing evidence of central nervous system symptoms, but no isolation of virus was made.

Two patients (V5-6443, OC and V5-6444, McA) were seen at the 155th Station Hospital. Both showed acute conjunctivitis. One (OC) also had a gingivostomatitis, skin rash, rhinitis, and ulcerated lesion at penile urethral orifice. A tentative diagnosis of Stevens-Johnson syndrome (44) was considered, but specimens were collected from both patients in an attempt to isolate a herpes virus. Fluids aspirated from the fornix conjunctivae were placed on the scarified cornea of rabbits. Fluids and tissue from a palatine bullae of OC was also inoculated intracerebrally into white mice. All attempts at isolation of a herpes virus were unsuccessful.

PSITTACOSIS-LYMPHOGRANULOMA VENEREUM GROUP: Aspirates from two cases of inguinal adenitis (V5-4474 and V5-3580) were received with requests for isolation and identification of the etiological agent. The aspirates were treated with penicillin and streptomycin to reduce bacterial contamination and then inoculated into the yolk sac of developing chick embryo. Yolk sac smears (24 to 48 hours after inoculation) stained by Giensa stain showed elementary bodies morphologically resembling those of thepsittacosis-lymphogranuloma venereum type.

Submitting installations have been notified to submit paired serum specimens to allow serological confirmation of diagnosis rather than to request attempted isolation of this agent in the future.

Department of Medical Zoology

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